Risk factors for cardiovascular disease and osteoporosis in adolescent Argentinian women with special reference to anorexia nervosa

Matzkin, Valeria

Risk factors for cardiovascular disease and osteoporosis in adolescent Argentinean women with special reference to Anorexia Nervosa

By Valeria Matzkin

A thesis submitted to the University of London for the degree of Doctor of Philosophy in the Faculty of Science

July 2004

Department Nutrition and Dietetics

Franklin Wilkins Building

150 Stamford Street

London SE1 9NN

Dedicado a mis padres

(To my parents)

Acknowledgements

I would like to thank:

Professor Catherine Geissler for supervising and guiding me throughout my PhD, and Dr. Moira Taylor for her early and valuable input on my work. Dr. Slobodianik, my supervisor in Argentina, for offering support and enthusiasm when I most needed it. Dr. Anabel Pallaro, Dr. Susana Feliu, Bioquímica Inés Fernandez and Bioquímica Maria Fernanda Godoy, from the University of Buenos Aires, for their contribution in my research project. Dr. Mabel Bello for allowing me access to the ALUBA and Dr. Roberto Eggía and Dr. Marcela Caretti for facilitating the work with patients with eating disorders. Dr. Raúl Coniglio, for sharing his vast knowledge on lipids and for his contribution in my research project. Dr. Pablo Mokfalvi and Dr. Viviana Mastreta from Central Lab for carrying out the blood analyses in Argentina. Robert Gray, Hannah Theobald, Sarah Berry and Zoue Lloyds-Wright for helping me with the blood analyses in London. Janes Thomas and Dr. Richard Shiow for their helpful feedback on my work. Professor Tom Sanders for his valuable advice on lipids and for facilitating the use of his lab. Profesor Ismael Pradas and Licenciado José Luis Sampaulo from the Ort school, Headminister Norma Colombato and Profesora Marta Castellan from the Emem 4 school, and Headminister Norma Camicia and Profesora Magdanela Casagrande from the Comercial 12 school. Ruth Boyd for checking the English of this thesis and for sometimes guessing the meaning of my thoughts. Licenciada Gabriela Zolotnik and Licenciada Florencia Pecenko for collaborating with the anthropometric measurements in the schools. Simon for always being there, giving me confidence, understanding and optimism. My family, for their support and encouragement along the way; without them this thesis and all the knowledge and experience I gained from it would not exist. I am especially grateful to all the schoolgirls, the professionals working in the eating disorders institutions, the control subjects and the patients who volunteered to take part in the study.

Abstract

This thesis examines: dietary and behavioural patterns in adolescents with reference to eating disorders and the risk for cardiovascular disease and osteoporosis; cardiovascular risk factors in patients with Anorexia Nervosa (AN); and whether these are considered during treatment. Little research in this field exists in Argentina, where there is an exaggerated desire for thinness. Four studies were performed:

Dietary and behavioural patterns in adolescents were investigated in 15-17 year old schoolgirls (N=342) from three different socio-economic (SE) groups. 62% of the girls wanted to lose weight and 40% were dieting, especially in the upper SE girls (58%). Body Mass Index was greater (22.4 kg/m^2, SD 3.1) in dieters than in non-dieters (20.3 kg/m^2, SD 2.1). 37% of the low SE girls smoked tobacco and 39% consumed alcohol, compared with 19% and 28% of the upper SE girls. Mean fat and carbohydrate intakes were 39% and 46% of the total energy. Many of these patterns indicate an increased risk of AN, cardiovascular disease and osteoporosis in adolescents.

Clinical Notes Review: documented cholesterol concentrations, on admission and at follow up, between patients with AN (N=308) and an age and sex-matched healthy Argentinean population (N=216) were compared. Patients had significantly higher cholesterol and LDL on admission, placing them at greater cardiovascular risk. Values had decreased to normal after nine months of treatment for AN.

Follow up Patients with AN: biochemical risk factors for cardiovascular disease were compared in patients with AN on admission and after four months of treatment (N=30) and controls (N=30). Cholesterol, LDL and Apo B concentrations were significantly higher in patients on admission, while retinol and tocopherol were lower. At follow-up most elevated biochemical parameters had decreased, but not significantly.

Treatment Assessment: institutions (N=13) were surveyed to assess treatment for AN. Cardiovascular disease was not recognised as a long-term problem and a high fat diet to achieve weight gain was prescribed by 61%. Allowance of behavioural choices related to cardiovascular disease and osteoporosis (e.g. coffee, tobacco, alcohol and vegetarianism) differed by institution.

These results suggest that preventive measures are needed to target the potential risk of AN, cardiovascular disease and osteoporosis in adolescents. As patients with AN are at greater risk of cardiovascular disease and osteoporosis, these need to be addressed in treatment.

Table of Contents

Table of Contents **continue...**

Detailed Table of Contents

Detailed Table of Contents continue...

Detailed Table of Contents continue...

List of Tables

page no.

List of Tables

continue...

page no.

Chapter 3

List of Tables

continue...

page no.

Chapter 4

List of Tables

continue...

page no.

Chapter 5

Appendix 1

List of Graphs

page no.

Chapter 2

Chapter 3

Chapter 4

Chapter 5

List of Figures

List of Appendices

List of Appendices

continue...

page no.

Chapter 4

Chapter 5

List of Abbreviations

ACAT	Cholesterol Acyltransferase
ACTH	Adrenal Corticotrophin Hormone
AdoMet	5-Adnosylmethionine
ALUBA	Association against Bulimia and Anorexia
AN	Anorexia Nervosa
Apo	Apolipoprotein
BMI	Body Mass Index
BMD	Body Mass Density
BMR	Basal Metabolic Rate
BN	Bulimia Nervosa
cm	centimeters
CM	Chylomicron
CRF	Corticotrophin Releasing Factor
DHA	docosahexaenoic acid
DPA	docosapentanoic acid
DSM IV	Diagnostic American Psychiatry Association
EFA	Essential Fatty Acids
EPA	Eicosapentanoic acid
EI	Energy intake
FSH	Follicle Stimulating Hormone
g	grams
GH	Growth Hormone
GTT	Glucose Tolerance Test
HDL	High Density Lipoprotein
HMGCoA reductase	Hydroxymethylglutaryl Coenzyme A reductase
HTGL	Heparin Hepatic Triglyceride Lipase
IDL	Intermediate Density Lipoprotein
IGF-1	Insulin-like Growth Factor 1
kcal	kilocalories
kg	kilograms
LCAT	Lecithin-Cholesterol Acyl Transferase

LDL	Low Density Lipoprotein
LH	Luteinising Hormone
Lp (A)	Lipoprotein A
LPL	Lipoprotein Lipase
m	meter
mg	milligrams
mm	millimetres
mmHg	millimetres of mercury
mmol	millimoles
MTHF	5-Methyltetrahydrofolate
MUFA	Monounsaturated Fatty Acids
N	Number of subjects
NCEP	National Cholesterol Education Program
PSF	Polyunsaturated Fatty Acids
PVD	Peripheral Vascular Disease
RBP	Retinol Binding Protein
SD	Standard Deviation
SFA	Saturated Fatty Acids
T3	Triiodothyronine
T4	Thyroxine
TC	Total Cholesterol
TG	Triglycerides
THF	Tetrahydrofolate
TRH	Thyrotrophin Releasing Hormone
TSH	Thyroid Stimulating Hormone
VLDL	Very Low Density Lipoprotein
WHO	World Health Organisation

Chapter 1 - Introduction

Chapter 1 - Introduction

Cardiovascular disease and osteoporosis are major world public health problems, being among the main causes of morbidity and mortality in women. Dissatisfaction with weight, slimming and the adoption of unhealthy methods to lose weight such as very low energy diets, imbalanced vegetarian diets, tobacco smoking and excessive coffee and alcohol intake are common features in young women. This thesis aims to establish whether these dietary and lifestyle behaviours, in pursuing thinness, may predispose a higher risk for future eating disorders, cardiovascular disease and osteoporosis. Anorexia Nervosa (AN) is an eating disorder which often begins in early teens affecting girls who are over-preoccupied with body shape. During the course of AN and as a result of starvation, many metabolic and behavioural adaptations occur. One of these adaptive changes includes hypercholesterolemia, which is a known major risk factor for cardiovascular disease. There is also a high risk of osteoporosis, with many patients showing reduced Bone Mass Density (BMD). This increased risk for cardiovascular disease and osteoporosis is rarely recognised in the management of patients with AN and, in fact, patients are often advised to follow a high fat diet to rapidly recover weight.

Argentina has a high prevalence of cardiovascular diseases; however few studies have been carried out in this area. In a comparison of international death rate in 1978, Argentinean women from all ages had one of the highest cardiovascular mortality rates (155.2/100,000) (Haugeer-Klerene and Balossi 1987). Mortality from cardiovascular disease significantly increased in women aged 34-44 years from the 1960 to the 80s. Although there was a tendency for the mortality rate from coronary heart disease to decrease from 1980 to 1997, this was more significant in males than in females (Sosa Liprani et al 1999). Because of the elevated incidence of heart diseases in middle aged Argentinean women (30 to 49 years) (INDEC 1997) and knowing that this begins at young ages (Mcnamara et al 1971), there is a need to detect individuals at risk prior to the presentation of clinical signs and symptoms.

Possible explanations for the raised mortality rate are the high prevalence of hyperlipoproteinemia (Coniglio et al 1992) and the high consumption of saturated fat.

Beef intake was estimated as 58 kg/capita/year in 1998 (Avery 1998), compared with 16.6 kg/capita/year in the UK in 1997 (Fapri-Ireland Partnership Economic Projections for Irish Agriculture). Another factor that may contribute to the high mortality rate is the greater use of tobacco, particularly in young females, compared with other Latin-American countries (PAN 2002). Argentinean women, therefore, are especially vulnerable to cardiovascular disease.

An Argentinean study carried out by Garcia and co-workers (1997) showed that 84% of 1,338 females between 10 to 20 years were dissatisfied with body weight, which may lead to body discontent and eating pathologies. Bello (1995) applied the Eating Attitude Test (EAT), which is a questionnaire measuring eating disorder symptoms, in secondary schools from Buenos Aires and observed that 29% of schoolgirls scored positively in the EAT and 9% fulfilled the criteria for an eating disorder. Since most epidemiological studies on eating disorders have been carried out in more developed societies, there are not other statistics on the incidence of eating disorders in Argentina. However, there is evidence to suggest that eating disorders are highly frequent (Zuckerfeld et al 1998a; Meehan and Katzman 2001).

Aims of the thesis

This thesis aims to study dietary and behavioural patterns in adolescent girls related to the risk of eating disorders and potential future cardiovascular disease and osteoporosis. It also aims to investigate cardiovascular risk factors in patients with Anorexia Nervosa (AN) and review whether treatment programmes for AN address the potential risk for cardiovascular and osteoporosis. Five studies, presented in the following chapters, were carried out to address all issues discussed above.

Chapter 2 (Study 1) - Risk factors for cardiovascular disease and osteoporosis in adolescent woman. This study aims to investigate the dietary intake, tobacco smoking, body dissatisfaction, alcohol and coffee intake, exercise practice, dieting behaviours and other lifestyle habits related to eating disorders, cardiovascular and osteoporotic risk in schoolgirls from three different socio-economic groups. Whether

patients with AN also exhibit risk factors for cardiovascular disease is discussed in the following studies.

Chapter 3 (Study 2) - Clinical notes review. This study aims: 1. To compare documented total blood cholesterol concentrations in patients with AN on admission with Argentinean reference samples, 2. In patients, to compare documented admission and follow-up cholesterol concentrations and to correlate cholesterol concentrations with the age of the patients, the use of tobacco and self-induced vomiting. The next studies investigate other risk factors for cardiovascular disease in a selected group of patients with AN.

Chapter 4 - Follow up patients with AN (Study 3 and 4). These studies aims to observe the impact of recovery on biochemical risk factors for cardiovascular disease (lipid profile, homocysteine, glucose, vitamins, essential fatty acids, fibrinogen and hormones), using a newly diagnosed group of patients with AN and a sex and age-matched control group. Whether treatment programs for AN address this risk is explored in the next study.

Chapter 5 - Treatment assessment (Study 5). This study aims to review whether the institutions treating AN in Argentina consider patients with AN to be at risk for cardiovascular disease and osteoporosis, and whether advice during treatment tending to reduce the risk for these future diseases is provided.

The following section 1.1 reviews background data on AN including definition, epidemiology, aetiology and causes of death.

1.1 Anorexia Nervosa (AN)

1.1.1. Definition, epidemiology and aetiology

Anorexia nervosa (AN) is a psychiatric disorder characterised by a voluntary drastic reduction of food intake and consequent weight loss, extreme hyperactivity and abnormal thoughts and attitudes towards body weight, body shape, food and eating. The disorder was first described in France by Morton (1764), followed by many others (Marce 1860; Lasegue 1873; Gull 1874). However, the existence of fasting, self-starvation and purges had been known for centuries (Van Deth and Vandereyecken 1988). The criteria for the diagnosis of AN as described by American Psychiatric Association (DSM-IV 1994) are as follows:

I. Refusal to maintain body weight at or above the minimally normal weight for age and height (i.e. Weight loss leading to maintenance of body weight less than 85% of that expected, or failure to make expected weight gain during period of growth, leading to body weight less than 85% of that expected).

II. Intense fear of gaining weight or becoming fat, even though underweight.

III. Disturbance in the way in which one's body weight or shape is experienced, undue influence of body weight or shape on self evaluation, or denial of the seriousness of the current low body weight.

IV. In postmenarchal females, amenorrhea, i.e. the absence of at least three consecutive menstrual cycles (A woman is considered to have amenorrhea if her periods occur only following hormone, e.g. oestrogen administration).*

The diagnosis discriminates between specific types:

Restricting or non purgative type: during the current episode of anorexia, the person has not regularly engaged in binge-eating or purging behaviour (e.g. self induced vomiting or misuse of laxatives, diuretics or oedemas).

Non restricting or purgative type: the person has regularly engaged in binge-eating or purging behaviour.

* Note: For the full diagnosis all the criteria points are required. The diagnostic criteria includes amenorrhea as one of the symptoms of AN. However, the concept of 'remission' is also introduced, which means that the patient does not necessarily have to suffer from amenorrhea at the time of examination to be diagnosed with AN because it might have occurred prior to clinical presentation.

Risk factors for eating disorders

The aetiology of AN is uncertain. The current view is that AN arises from a combination of long-standing genetic, familial, psychological and social conditions. The studies of risk factors for eating disorders are still in formative years, as many of the risk factors are not yet well established, although some of the recognised risk factors are shown below.

Sex: eating disorders are 40 times more prevalent in women than men (Turnbull et al 1996).

Genetic and family predisposition: Garfinkel and Garner (1982) found a greater concordance of AN in monozygotic twins than in dizygotic twins, suggesting a genetic link. Furthermore, psychiatric disorders (affective disorder, AN, BN, alcoholism, etc) are more common in first degree relatives of patients with eating disorders.

Age: AN usually develops in early adult life, between puberty and the age of 25 years. An age of onset before 25 years old was formerly one of the diagnostic criteria (Feighner et al 1972), but it is now recognised that it can occur in older men and women.

Psychological characteristics: Feelings of inadequacy, depression, anxiety, loneliness and fear of interpersonal relationships may contribute to the development of an eating disorder. Searching for parental independence, social recognition and low self-esteem are all stress generating states in adolescents. In girls with AN, the obsession with the body image and food may serve as a means of coping with this stress, facilitated by a personality with obsessional and perfectionist traits, and by modern culture, with its unrelenting idealisation of thinness.

Body dissatisfaction: promotes dietary and compensatory behaviour focussed on a compulsion to lose weight. It also encourages negative affect. Some people binge-eat in order to provide comfort and distraction from adverse emotions. Subsequently,

vomiting following overeating would be used as an emotional catharsis, as well as providing an effective means of preventing weight gain.

Socio-cultural pressure for thinness: promotes body dissatisfaction, which in turn places individuals at risk of dieting and eating disorders. Pressure to be thin from family, peers and media contributes to internalisation of the ideal. Athletes and ballet dancers showed a higher incidence of eating disorders (Garner and Garkinkel 1978) because of a greater pressure to maintain a low body weight. Also, body dissatisfaction tends to be greater in Western than in non-western cultures (Furnhan and Alibhai 1983).

Data from the UK suggest that the incidence of AN as estimated from diagnosis by general practitioners was 4.2/100,000 population in 1993 (Eating Disorders Association (EDA) 2003). However, more recent data estimated the incidence at 34.1/100,000 in the age group of 10-19 years (EDA, 2003). Far more common than AN is bulimia nervosa (BN). Button (1990) estimated that 5-10% of post-pubertal girls and women suffer from an eating disorder or borderline condition. This is probably a high estimate; a more realistic estimate is in the order 1% (Johnson-Sabine et al 1988).

The awareness of eating disorders has increased in the last few decades, which may have inflated numbers because of better diagnosis ascertained. The incidence of the admissions to psychiatric institutions in Denmark increased from 0.42 per 100,000 in 1970 to 1.36 per 100,000 in 1988 (Moller-Madsen and Nystrup, 1992). Sullivan (1995) claimed that AN represented one of the most common chronic illnesses in teenage girls and it has one of the highest rates of mortality for any psychiatric condition. The aggregate annual mortality rate associated with AN (0,0054) was more than 12 times higher than the annual death rate due to all causes of death for females 15-24 years old in the general population (0,00045 deaths per year), and more than 200 times greater than the suicide rate in the general population (0,00002 suicides per year).

AN is a distinctive illness because the biological disturbances of patients with AN are different from those seen in individuals who are starved. For example, the altered perception of hunger and appetite, the disturbances in body perceptions, the hyperactivity, the hypercholesterolemia, the hypercarotenemia and the electrolyte imbalance are typical only of AN and absent in starvation.

In the process of self-starvation body fat is diminished, resulting in hypersensitivity to cold and heat. The metabolism slows to accommodate the reduced food intake, as is observed in people suffering from famine. Important physical features of AN are the presence of lanugo (fine hair) over the trunk, face and arms, dry skin, cold and blue extremities, low blood pressure and arrhythmia, muscle weakening, cessation of menses, constipation and dental erosion from repeated vomiting. A large number of medical complications have been documented in patients affected by AN; these include menstrual, reproductive, gastrointestinal, renal, metabolic, dermatological, bone, neurological and cardiovascular abnormalities. In the majority of the patients the symptoms of these complications disappear with appropriate nutrition and weight restoration. However, other complications such as oesteoporotic changes may leave permanent effects particularly in teenage girls where there is a critical period of bone mineralisation (Sharp and Freeman 1993).

Addictive behaviours in AN

Addictive behaviours are common among patients with eating disorders, since food deprivation may lead to drug abuse. Wiederman and Pryor (1996) found that 14% of patients with AN and 30% of patients with BN were tobacco smokers. Several authors have observed that non-restricting type patients and patients with BN are more likely to use addictive substances (tobacco, alcohol, caffeine, drugs, etc) than the restricting type AN patients (Garfinkel et al 1980; Toner et al 1986; Frank et al 1987; Haug et al 2001).

Tobacco smoking is one of the major modifiable risk factors for cardiovascular disease (Slone et al 1978; Wood et al 1998) and osteoporosis (Law et al 1997). Smoking induces atheroclerotic plaque formation in adolescents and adults (PDAY

1990) and increases oxidative stress. Tobacco smoking also promotes changes in lifestyle risk factors associated with osteoporosis such as, lower than average body weight, earlier average of menopause, alcohol consumption and low micronutrient intake.

Substance abuse has also been noticed in young people who diet to lose weight. Secondary school students who engage in weight control behaviour are more likely to use marihuana, tobacco and alcohol (Killen et al 1987; Frank et al 1987; Neumark-Sztainer et al 1998). Alcohol has a J shaped relationship to the mortality risk for all causes in middle-aged and elderly subjects (Rimm et al 1999). Moderate alcohol intake has been shown to increase blood HDL values, and red wine has been shown to inhibit oxidation of LDL in vitro (Frankel et al 1993). On the other hand, excessive alcohol drinking (more than 3 units/day) has been linked to cardiovascular disease because it increases blood pressure, triglycerides and decreases lp(a) (Moreira et al 1998; Nanchahal et al 2000). Heavy and regular alcohol intake is also an established risk factor for osteoporosis (Moniz 1994).

In the battle to reduce food intake, many patients with AN consume large quantities of drinks containing caffeine (coffee, tea and diet Coca-Cola), which provide few calories and can suppress the appetite. Sours (1983) describes the cases of two women with AN who abused caffeine beverages. Forman and colleagues (1997) reported the extreme case of a patient with non-restricting AN who ingested 20g of caffeine in a suicide attempt, resulting in infarction. Excessive coffee intake has been associated with an increased incidence of cardiovascular disease (LaCroix et al 1986; Grubben et al 2000). Zock and co-workers (1990) showed that the lipid-rich supernatant of boiled coffee obtained after centrifugation raised serum LDL and blood triglyceride concentrations. They suggested that a cholesterol-raising agent be contained in the lipid fraction. More recently, Heckers and other investigators (1994) demonstrated that the diterpenoid coffee constituents cafestol and/or kahweol are the active cholesterol-raising substances. Excessive caffeine intake has been associated with decreased bone density, since it promotes calcium excretion (Hernandez Avila et al 1991).

Energy restriction

Early descriptions of food pattern in AN mention a specific avoidance of carbohydrates (Crisp 1967; Russell 1967; Garfinkel and Garner 1982). However, high fat foods have been mistakenly described as high carbohydrate items because of their sweetness (Rock and Curran-Celentano 1994). In contrast, Thibault and Roberge (1987) demonstrated that instead of avoiding carbohydrates, patients maintained their total carbohydrate intake but rejected simple carbohydrates.

Other studies suggest that reduced energy intake (EI) is most often achieved by a particular avoidance of dietary fat rather than dietary carbohydrates. Mordasini and colleagues (1978) observed that the diet of individuals affected by AN is characterised by a low intake of energy and fat and a high carbohydrate intake. Beaumont and co-workers (1981) evaluated the intake of patients with AN at two stages of the illness: when they start dieting (onset of AN) and at the time of greatest emaciation. At the period of most severe emaciation carbohydrate consumption was increased while that of protein and fat was decreased. Rather than avoid carbohydrates, patients appeared to reject foods with a high-energy density (rich in fat).

Besides the dilemma of carbohydrate or fat phobia, a consistent finding is the low EI, which is illustrated in Table 1.1. Vegetarianism, in its various forms, has been reported in AN by several researchers (O' Connor et al 1987; Bakan et al 1993 and Hadigan et al 2000). O'Connor and colleagues (1987) associated the avoidance of red meat with the lowest BMI in a sample of patients with AN, suggesting that such patients constituted the most severely affected group. Meat refusal usually sustains the low fat and EI. Low EI facilitates nutritional deficiencies, such as low antioxidant vitamin and mineral intake, and it has been hinted as a risk factor for future cardiovascular disease and osteoporosis. Lapidus and collaborators (1986) carried out a massive longitudinal study with women in Sweden. They observed that energy intake was inversely correlated to the 12-year incidence of myocardial infarction and concluded that sub-optimal intake of nutrients may be an important factor in the pathogenesis of ischemic heart disease. An alternative explanation is that overweight

and obese subjects under-report food intake. Food restriction also leads to inadequate calcium intake and low body weight, which are linked to osteoporosis.

Some researchers (Morgan and Russell 1975, Windauer et al 1993 and Nova et al 2001) reported that a large number of patients continued to restrict their food intake at long-term follow up. This suggests that some treatments are ineffective at improving the patients' diet adequacy, even after discharge.

Table 1.1: Energy and macronutrient intake reported in studies of patients with AN

Author and year of study	N	EI (Kcal/d)	Carbohydrate (% EI)	Protein (% EI)	Fat (% EI)
Russell (1967)	9	1031	33	18	49
Kanis et al (1974)	24	911	35	19	55
Halmi and Fry (1974)	12	944	47	22	32
Gwirtsman et al (1989)	24	1017	53	16	30
Thibault and Roberge (1987)	25	773	45	17	36
Moreiras-Varela et al (1990)	48	1410	44	19	37
Philipp et al (1988)	8	2157	45	15	35
Schreiber et al (1991)	13	1983	40	14	43
Obarzanek et al (1994)	10	1105	56	19	25
Hadigan et al (2000)	30	1289	69	15	16
Nova et al (2001)	14	1155	51	22	27

Outcome of AN

There is general agreement that AN carries a great variability in outcome. The disorder may be presented as a single illness in adolescence, it may attenuate over time, become a chronic disorder or even ultimately result in death. It is estimated that about 50% of patients recover after undertaking treatment, 30% retain partial symptomatology and 20% become chronic. Cremerius (1978) studied a small sample (n=13) of German subjects with AN and found that 1/3rd remained with the symptoms, 1/3rd became worse and 1/3rd showed improvement after an average of 20 to 25 years of follow-up. Outcome is variable in patients with AN. Herzog and colleagues (1997) reported that recovery at follow-up in long-term outcome studies ranges from 17 to 77%. In AN there is a high rate of premature termination of treatment, as Vandereyck and Pierloot (1983) reported a drop-out rate of about 50%. The authors associated dropping out of treatment with increased age, duration of the

illness, low level of education, social class and treatment method. According to Dally and Sargent (1966) patients are thought to have reached a state of chronicity if they continue to display symptoms after five years despite suitable treatment. AN, therefore, can be a chronic illness with high incidence of relapses, exacerbation and syndrome shifts.

1.1.2. Causes of death in Anorexia Nervosa (AN)

Mortality rate from all the causes of death in AN varied from 0 to over 20% and it increases as the observation period lengthens. Morgan and Russell (1975), for example, observed a mortality of 3% after 5-6 years follow-up and of 15% after 20 years (Ratnasuriya et al 1991). Theander (1985) in Sweden found one of the most elevated crude mortality rates of 18%, after a mean observation time of 33 years, making AN the most lethal of all psychological disorders.

In general, followed-up patients showed a higher incidence of physical illnesses than the normal population. Deter and Herzog (1994) found a fourfold increase in somatic morbidity at follow up. Psychiatric symptomatology persists in the majority of the studies at follow-up (Hawley 1965; Schwartz and Thompson 1981; Herzog et al 1988; Rosenvinge and Mouland 1990; Norring and Sohlberg 1993; Deter and Herzog 1994; Eckert et al 1995; Herpertz-Dahlmann et al 1996; Herzog et al 1997). Osteoporosis, loss of fertility and renal complications are among the most common co-morbidities. Loss of fertility is one of the main complications of AN. Besides the lack of sexual interest and poor marital relations, the incidence of pregnancy is low, with the majority of patients (64%) being childless at 12.5-year follow-up (Brinch et al 1988). Amenorrhea or oligomenorrhea are central features of the illness, with some patients presenting these symptomatologies even after discharge from treatment.

Causes of death in AN are rarely reported, since it is very common to find the diagnosis 'complication of the illness' as the cause of death without any further details. Suicide has an important incidence in AN and suicide rates are similar to those observed for schizophrenia (Moller-Madsen et al 1996).

Cardiac complications in AN are by far the most common, and the most likely to result in fatalities. These include bradycardia, tachycardia, hypotension, ventricular arrhythmia (ectopic atrial rhythm, nodal escape beats and ventricular ectopy), cardiac failure and sudden death (Sharp and Freeman 1993). Acid-base and electrolyte imbalances are commonly observed in subjects with AN (Zwaan and Mitchell 1993), which can lead to severe cardiac arrhythmia and other electrocardiographic abnormalities. Reduction in cardiac dimensions has been described in normal patients on semi-starvation diets (Keys et al 1950) and in patients with AN (Moodie 1987). The small volume of the left ventricle, due to exaggerated weight loss (Moodie 1987 and De Simone et al 1994), may contribute to the abnormal response to exercise, and perhaps it is a factor predisposing to sudden cardiac death. These types of cardiac deaths are completely unrelated to lipid deposition and cardiovascular disease.

Cardiovascular disease in subjects suffering from AN is usually not expected because these patients are generally very young. However, there have been cases of sudden death due to myocardial ischaemia in young patients with AN. Garcia-Rubira and co-workers (1994) reported the case of a 39-year-old patient with AN, who died from myocardial infarction during re-feeding. She was a heavy cigarette smoker and presented hypercholesterolemia. Isner and colleagues (1985) reported the case of three patients with AN (29, 32 and 37 years old) who died suddenly. Sudden death is defined as the sudden and unexpected occurrence of death, for which no satisfactory explanation can be ascertained. In all three cases, extensive lipofuscin deposits and myocardial impairment were observed. Moesli (1967) reviewed twenty-two cases of AN from the literature and three of his own, and noticed a high frequency of cardiac and circulatory complications. He reported the death of a 29-year-old woman, who had had a 10-year history of AN. Autopsy revealed myocardial infarction due to an isolated atherosclerotic plaque.

Chikause and colleagues (1988) described the case of a 37-year-old deceased patient with AN. Histological analysis found mild atrophy and mild oedema in the cerebrum, extreme atrophy in the cardiac muscle and moderate deposition of lipofuscin granules in the myocardial cells. There has also been documentation of

patients suffering from atherosclerosis related diseases such as diabetes, hypertension, goitre, marked varicosis (Herzog et al 1997), gangrene and occlusion of the femoral arteries (Pasternack 1970).

From the preceding facts, it is possible to conclude that AN can be a lethal disorder. Patients are exposed to a greater number of risk factors for future cardiovascular disease and there is growing evidence of cardiovascular disease morbidity and mortality among AN sufferers. There is also a lack of longitudinal studies investigating causes of death in subjects who had AN 40 to 50 years ago or examining the incidence of cardiovascular disease in recovered patients. Although cardiovascular disease is not the main cause of death in AN, its seriousness is underestimated in the treatment because of its long-term impact on health. Cardiovascular risk should be addressed in the treatment and prevention of the illness.

Health risks associated with AN include death and chronic diseases such as cardiovascular disease and osteoporosis. Causes of death are covered in section 1.1.2. Background information on cardiovascular disease, which is the main focus of this thesis, is given in section 1.2.1 and a brief background of osteoporosis in section 1.2.2.

1.2. Health risks associated with AN

1.2.1. Cardiovascular disease

1.2.1.1. Definition

Cardiovascular disease is a generic term, which includes coronary heart disease (CHD) (also known as ischaemic heart disease), stroke and peripheral vascular disease (PVD). A common feature of CHD, occlusive stroke and PVD is atherosclerosis of the large arteries. The current view of pathology of atherosclerosis is that it results from a response to vascular injury and one of the earliest signs is the presence of fatty streaks in the intima of large arteries. These fatty streaks consist of clusters of foam cells, which are macrophages laden with lipid, mainly cholesterol derived from LDL. Fatty streaks are evident in early life and many regress; however some progress to form fibrous plaques which are lipid poor. The fibrous plaque can undergo a series of proliferative changes eventually growing into a large occluding plaque. Cardiovascular events arise when plaques rupture triggering thrombosis or when the size of the plaque impairs blood flow resulting in ischaemia. The atherogenic process usually develops over several decades. Clinical symptoms are only evident when the blood supply to the tissue cells is impaired and the tissues suffer from a lack of oxygen and nutrients. If the tissue is the heart muscle, this can lead to chest pain on exertion (angina) and irregularities in the heartbeat (arrhythmia). If the occlusion of the coronary arteries is severe this will result in death of the oxygen-starved tissue (infarction). Temporary occlusion of the coronary artery followed by reperfusion results in ventricular arrhythmia and these can lead to sudden cardiac death. Occlusion of blood supply to the brain leads to cerebral infarction (stroke).

Mechanism of cardiovascular damage

Atherosclerosis

There are several mechanisms by which initial arterial injury can be caused but it is more likely to occur in areas where there is hemodynamic turbulence such as at the bifurcations of arteries. The risk is exacerbated by hypertension. Infection by viruses or bacteria, increased exposure to reactive oxygen species and other noxious chemicals, and elevated cholesterol concentrations have been shown to cause endothelial dysfunction and disruption. Endothelial damage permits monocytes to become trapped in the sub-endothelial layer and be converted into macrophages. Macrophages do not take up native LDL but can avidly take up modified LDL. These macrophages have scavenger receptors, which allow them to recognise and remove oxidised lipoproteins. PUFA in LDL can become oxidised or glycosylated, causing changes in the conformation of apolipoprotein B so that the protein is not longer recognised by the cell receptor and is taken up by macrophages. Macrophages can become overloaded with oxidised LDL and form 'foam cells'. When the foam cells die, the deposition of the lipid on the artery wall takes place, resulting in the formation of the atheriosclerotic plaque. Oxidized LDL also has toxic effects on the vascular endothelium and recruits inflammatory cells to the site of injury thus promoting the growth of the atherosclerotic plaque (Ross 1999).

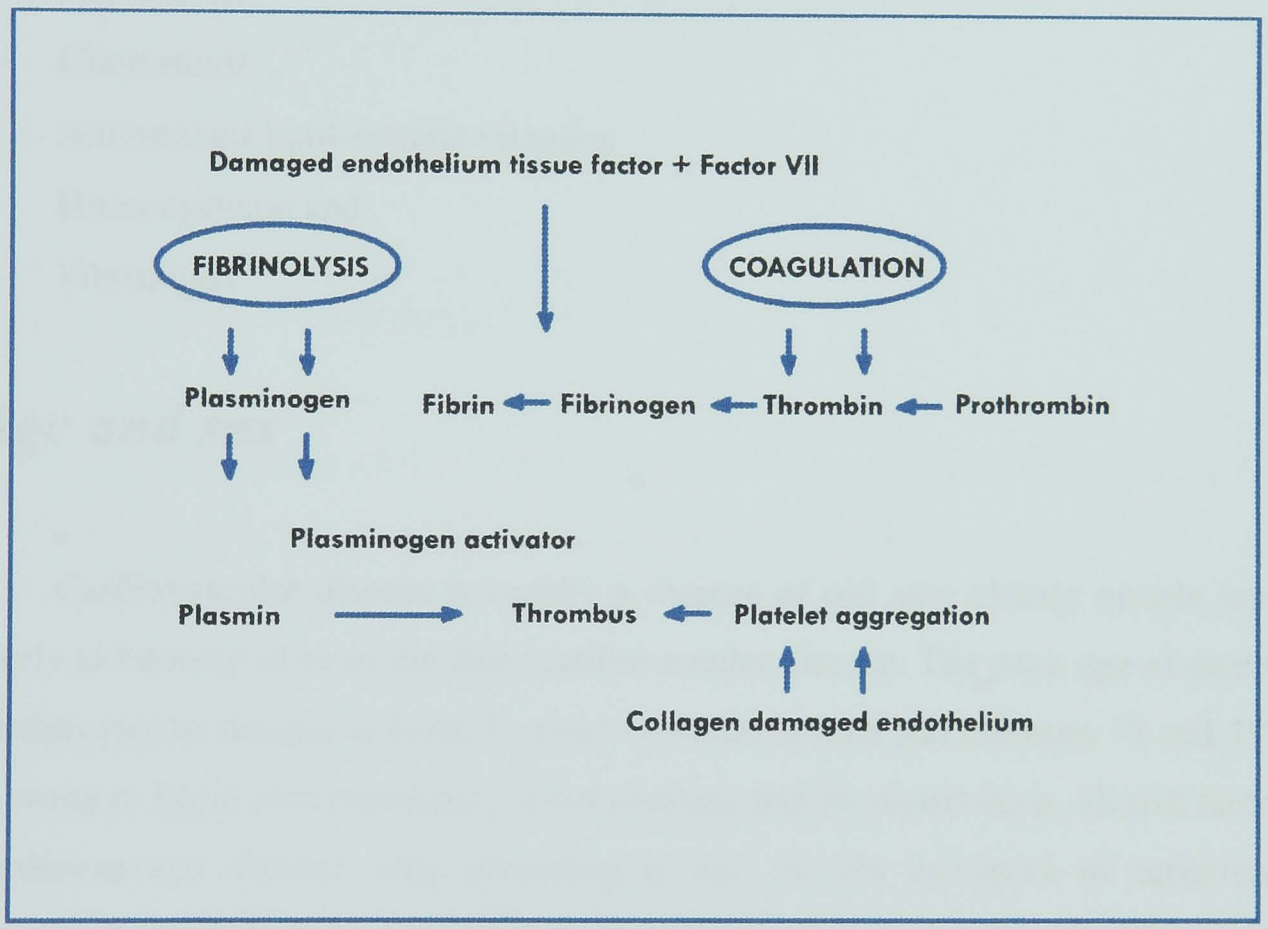
Thrombosis

Injury to the vascular endothelium can stimulate the cell membrane protein called 'tissue factor', which combines with the circulating coagulant factor VII. This complex initiates the cascade of reactions (the coagulation pathway) which ends with the generation of thrombin and deposition of fibrin at the site of injury. If this is accompanied by platelet deposition as a result of their activation by exposed collagen, this becomes a thrombus. The normal physiological role of a thrombus is to seal a wound. The fibrinolytic system originates the enzyme plasmin, which degrades the insoluble fibrin in the thrombus. The formation of a large thrombus in an artery already narrowed by fibrous plaque can obstruct the blood flow supplying the heart.

The lack of oxygen in the heart cells may result in a myocardial infarction. Low fibrinolytic activity has been linked to cardiovascular disease. High levels of factor VII and of fibrinogen may predispose the blood to clot and have been shown to predict cardiovascular disease (Figure 1.1).

Figure 1.1: Coagulation and fibrinolysis pathways

Source: Report of the Cardiovascular Review Group Committee on Medical Aspects of Food Policy (1995)



1.2.1.2. Risk factors for cardiovascular disease

The aetiology of cardiovascular disease is multi-factorial; it is caused by the interaction of different risk factors. A risk factor is a feature whose presence is related to an increased probability that a disease will develop later in time. The study of risk factors provides research areas targeted to the better understanding of the mechanism associated with the origin of the disease. When more than one risk factor is present the combined level of risk is increased; two or more risk factors multiply rather than

simply add to the effect. The isolated examination of a risk factor for cardiovascular disease is not possible, as factors tend to cluster together. Many of the lifestyle factors exert their effects through a number of physiological risk factors. For instance: BMI greater than 30, and particularly abdominal fat accumulation, can lead to increased insulin resistance, increased cholesterol and blood pressure. These physiological changes were initially originated by obesity, which is a factor caused by lifestyle.

Risk factors for cardiovascular disease are many, but only some will be discussed in this thesis. The selection of risk factors was made according to their relevance to AN. The risk factors to be reviewed are:

- Age and sex
- Cholesterol
- Antioxidant lipid-soluble vitamins
- Homocysteine and
- Fibrinogen

Age and sex

Cardiovascular disease is mainly a disease of old age; elderly people are more likely to become ill or to die from cardiovascular disease. The peak age of death from cardiovascular disease is from 70 years onwards in men and between 75 and 79 years in women. Lipid concentrations, blood pressure and fat distribution, all risk factors for cardiovascular disease, vary according to age. As the incidence of cardiovascular disease rises with increasing age, it is considered to be premature when it occurs before the age of 65.

Cardiovascular disease is more frequent in males than in females. The distinction is present at all ages, but is less after the menopause. A man is about 3-5 times more likely to suffer from cardiovascular disease than a pre-menopausal woman of the same age (Ashwell 1997). The incidence of cardiovascular disease death in females below the age of 54 is much lower than in males. Beyond this age the incidence rises and in females over 75 years old it approaches and overlaps levels seen in males. The cessation of the ovarian function in menopausal women may predispose them to

cardiovascular disease, since there is an increase in cardiovascular mortality in women following the menopause. Witteman and colleagues (1989) studied the aortic state by radiography in a sample of pre and post-menopausal women. They found aortic atherosclerosis in 3% of the pre-menopausal women and in 12% of the post-menopausal group.

Oestrogen deficiency is a common feature of AN, which may lead to hypercholesterolemia and be a risk factor for cardiovascular disease. Most of the patients are young at the time of presentation of the disorder and, therefore, they are not at risk of cardiovascular disease. However, these risk factors could be sustained during adulthod and became significant hazards for future diseases.

Cholesterol

Cholesterol as a risk factor

Several studies using different methodological approaches have demonstrated that high cholesterol concentrations are associated with cardiovascular disease. These include: epidemiological studies comparing cardiovascular disease and hypercholesterolemia incidence in different countries; prospective studies investigating the association of cardiovascular events and cholesterol values; studies on heart diseased patients and on patients with hypercholesterolemia, etc.

A 10% increase in LDL cholesterol is associated with an increase in the risk of cardiovascular disease of around 20% (Neaton et al 1992). Law and colleagues (1994) showed that the benefits of cholesterol reduction are related to age and, for example, a 10% reduction in cholesterol values produces a reduction in ischaemic heart disease of 50% at the age 40. Elevation of LDL cholesterol above 5.85 mmol/l (225 mg/dl) is usually due to genetic abnormalities or secondary causes, such as hyperthyroidism, renal or liver diseases. A cholesterol level of 5-6 mmol/l (190-230 mg/dl) may require drug therapy in a patient at high cardiac risk, whereas a cholesterol value of 7-8

mmol/l (270-310 mg/dl) may be left untreated in an individual at low overall risk (Wood et al 1998).

High concentrations of blood cholesterol have been widely reported in AN. Most of the studies observed high total cholesterol and LDL concentrations on admission and these levels tend to decrease gradually during treatment. However, an important factor with regard to risk is the duration of exposure to high blood cholesterol concentrations.

Previous studies assessing cholesterol levels in AN were examined in order to estimate the frequency of hypercholesterolemia. A search was conducted in the PUBMED of the National Library of Medicine to identify relevant studies. The entry data were unlimited in years and one of the inclusion criteria was that the papers were in English. Key words were 'cholesterol and AN'. Relevant references from these articles and from the review articles were also included. Those papers that only discussed BN, those whose sample sizes were smaller than five subjects, or review papers, were excluded. Searches were carried out from March 1999 to February 2003.

The results from the search are shown in Table 1.2. The review of previous studies in AN confirmed that high cholesterol values are a significant problem in AN. The main limitation for drawing conclusions from these studies is due to the different inclusion criteria used for the subjects.

Table 1.2: Previous studies reporting cholesterol values (mg/dl) in patients with AN

Author and year of study	Cholesterol					
	Patients			Controls		
	N	Mean	SD	N	Mean	SD
Crisp et al (1968)	37	297.4	85.6	37	228.8	55.7
Blendis and Crisp (1968)	8	310.0	100.3	R		
Mant and Faragher (1972)	5	183.8	69.7	R		
Halmi and Fry (1974)	12	214.0	44.0	R		
Nestel (1974)	5	318.7	85.0	R		
Kanis et al (1974)	19	244.0	21.0	R		
Mordasini et al (1978)	18	265.0	34.0	15	194.0	19.0
Halmi and Falk (1981)	40	187.9	43.9	R		
Bhangi and Mattingly (1981)	20	204.9	50.3	R		
Langan and Farrell (1985)	11	184.5	55.2	R		
Mira et al (1987)	90	215.8	50.0	30	193.8	28.8
Umeki (1988)	27	204.0	74.0	28	164.0	22.0
Van Binsberger et al (1988)	20	190.8	-	10	166.9	
Arden et al (1990)	16	168.0	49.0	16	161.0	22.0
Sanchez Muniz et al (1991)	12	198.5	32.3	9	193.8	18.5
Franssila-Kallunki et al (1991)	11	188.5	7.7	8	192.3	11.5
Uhe et al (1992)	10	200.0	7.7	6	146.0	7.6
Rock and Swendseid (1993)	9	148.3	36.7	18	171.1	25.0
Lewis et al (1994)	11	178.6	30.8	R		
Mehler et al (1998)	23	179.3	39.0	R		
Case et al (1999)	9	217.9	29.1	10	158.3	18.0
Feillet et al (2000)	14	208.0	38.2	R		
Boland et al (2001)	75	199.0	54.0	95	180.0	28.0
Total	**502**	**214.7***	**45.4**	**282**	**181.2**	**24.3**

R: Compared with reference values

T- test *p= 0.013

There are several studies following up patients with AN during treatment. The same Internet journal search described above was used to identify prospective studies, which assessed patients during treatment. Those studies not reporting baseline cholesterol levels were excluded. The result of this search is illustrated in Table 1.3.

Table 1.3: Previous studies reporting cholesterol concentrations (mg/dl) during treatment in patients with AN

Author and year of study	Cholesterol					
		Admission			Follow up	
	N	Mean	SD	Follow up period (months)	Mean	SD
Umeki (1988)	27	204.0	74.0	N/R	213.0	57.0
Arden et al (1990)	16	168.0	49.0	16.0	172.0	29.0
Halmi and Falk (1981)	40	187.9	43.9	1.0	192.9	32.8
Blendis and Crisp (1968)	8	310.0	100.3	N/R	283.7	67.0
Lewis et al (1994)	8	172.3	8.1	N/R	210.7	14.9
Casper et al (1980)	7	247.0	15.0	N/R	178.0	5.0
Kanis et al (1974)	11	261.0	34.4	10.0	218.0	10.6
Feillet et al (2000)	14	208.0	38.2	1.0	184.3	30.6
Total	**131**	**219.8**	**45.4**	**7.0**	**216.6**	**30.8**

N/R: not reported

T-Test

There have been many efforts to elucidate the causes of the hypercholesterolemia in AN. Case and colleagues (1999) found higher blood cholesterol and LDL levels in patients with AN who vomited compared with bulimics and controls. The authors suggested that decreased oestrogen activity might explain this difference, as the patients with AN were amenorrheic. Rock and colleagues (1996) related high blood cholesterol levels to frequency of dieting. They evaluated the nutritional status of undergraduate women and found that those who dieted were avoiding the consumption of dietary fat and had higher blood cholesterol values. It was suggested, therefore, that there might be a connection between avoiding fat intake and increasing blood cholesterol.

Feillet and co-workers (2000) found elevated lathosterol concentrations (a cholesterol precursor) in AN, indicating increased cholesterol synthesis. They also observed that high cholesterol level correlated with transthyretin (prealbumin), which is a marker of malnutrition and of protein-depleted states, indicating that the more malnourished the patients the higher the cholesterol values.

Cholesterol biosynthesis and regulation, and cholesterol absorption and metabolism will be discussed in Appendix 1.1 and 1.2

Factors affecting blood cholesterol levels

Thyroid hormones

The hypothalamic thyrotropin-releasing hormone (TRH) stimulates the release of the thyroid stimulating hormone (TSH) from the pituitary gland, which controls the release of thyroid hormones. The thyroid gland produces two major hormones: thyroxine (T4) and triiodothyronine (T3). The normal thyroid gland produces about 80% T4 and about 20% T3; however, T3 possesses about four times the hormone action of T4. In the circulation, 99% of the hormones are transported on proteins and the remainder is free. At least 80% of T3 and all reverse T3 (the inactive form of T3) results from the deiodination of T4 in the liver and peripheral tissues. Thyroid hormones have two major physiological effects: increasing protein synthesis and oxygen consumption.

Thyroid hormones, thyroid binding protein and TSH levels are usually abnormally low in patients with AN (Tamai et al 1986). Levels of T4 tend to be normal, while T3 levels are decreased. The low T3 concentrations probably result from a reduction in its peripheral conversion from T4 and an increased conversion of T4 to inactive reverse T3. Reduction in thyroid hormones in AN has the function of generally reducing metabolic rate and preventing nitrogen loss and muscle catabolism. Decreased thyroid hormones have also been observed in protein-energy malnutrition and in fasting obese individuals (Douyon and Schteingard 2002).

Thyroid hormones modulate LDL receptor, HMG CoA reductase and cholesterol 7α hydroxylase activity. Lack of thyroid secretion (hypothyroidism) is associated with elevated total and LDL cholesterol concentrations (Stone 1994; Feld and Dickey 2001). Consequently, many attempts have been made to link the deficient thyroid function with the hypercholesterolemia of AN.

Cortisol

Cortisol is a glucocorticoid hormone produced by the adrenal glands and tightly regulated by the hypothalamus. Cortisol plays an important role in permitting the body to cope with short-term stress. Some of the anti-stress defensive mechanisms of cortisol include the release of neurotransmitters, hypoglycemia by insufficient cell glucose, and anoxia by insufficient cellular oxygen. Cortisol suppresses inflammation and immune reactions through the inhibition of cytokines. Cortisol is also needed to maintain normal vascular integrity and the volume of body fluids. The metabolic effects of cortisol include promoting gluconeogenesis and protein breakdown, increasing plasma glucose, insulin and body fat.

Hypercortisolemia has been associated with hypercholesterolemia (Nanjee and Miller 1989) and cardiovascular disease (Colao et al 1999). Cortisol was also shown to be elevated in volunteers exposed to psychometric indices of stress (depression, anxiety and hostility) (Francis 1979), and this may indirectly contribute to the risk of cardiovascular disease. Furthermore, people with Cushing syndrome have increased risk factors for cardiovascular disease (Tauchmanova et al 2002). Cortisol stimulates both lipid accumulation and mobilisation and inhibits the antilipolytic effect of insulin. High cortisol levels increase blood cholesterol by effect on the LDL receptor, HMG CoA reductase and cholesterol 7α hydroxylase activity.

Cortisol has been extensively studied in AN using different experimental approaches. Patients with AN have been reported to have low urinary 17-ketosteroids (Garfinkel et al 1975; Warren and Vande Wiele 1973; Kennedy et al 1991). Despite this finding, high plasma cortisol was present in all these studies. Although cortisol levels are increased in AN, this occurs at the expense of decreased androgen levels. Zumoff and co-workers (1983) demonstrated a low ratio of dehydroepiandrosterone/cortisol. These two steroids are derived from a common precursor: 17-hydroxypregenenolone. The conversion of 17-hydroxypregenenolone to cortisol requires the enzyme 3-β-hydroxysteroid dehydrogenase, and the conversion of 17-hydroxypregenenolone to dehydropeiandrosterone needs the enzymes 17, 20 lyase. It has been hypothesised that in AN there is a low lyase activity and a high

dehydrogenase activity also seen in pre-pubertal children (Zumoff et al 1983). These enzymes are regulated at hypothalamic level.

Cortisol is tightly regulated by the hypothalamic release of adrenal corticotrophin hormone (ACTH), which triggers the pituitary corticotrophin releasing factor (CRF) to stimulate cortisol secretion by the adrenal cortex. Casanueva and co-workers (1987) challenged patients with AN by ACTH and the adrenal steroid products were monitored. ACTH induced the increment of blood cortisol, without this being regulated by CRF. This finding suggested that in AN there is an abnormal CRF production but ACTH function is conserved. In contrast, Gold and colleagues (1986) found that the administration of CRF in patients with acute AN increased plasma cortisol concentrations and produced a blunted ACTH response. Hotta and co-workers (1986) studied the pituitary-adrenocortical response to CRF injection and the cerebrospinal fluid concentration of CRF in patients with AN. Plasma cortisol levels and the CRF concentrations in the cerebrospinal fluid were higher in patients than in controls after the CRF injection. The ACTH response to the injection was lower in the group of patients. The researchers suggested two explanations for the low ACTH response to CRF: 1. Hypersecretion of CRF and; 2. Over-production of cortisol and impaired cortisol hepatic metabolism

Changes in cortisol production include a disturbance in the diurnal hormonal rhythm. In healthy subjects, blood cortisol concentrations are higher in the early morning hours and lower at night, which is also the diurnal variation of ACTH. Kennedy and co-workers (1991) observed significantly higher plasma cortisol concentrations in the morning at 6 am, but also in the evening at 9, 10, 11 and 12 p.m. in patients with AN in the emaciated state, compared with re-feeding and with controls. The elevation of cortisol levels during the early morning and in the earlier part of the night may reflect a reduced rate of cortisol breakdown, as a mechanism of energy conservation, and a reduced urinary cortisol excretion.

In conclusion, cortisol concentrations in the cerebrospinal fluid and blood of underweight patients with AN are elevated and decrease following weight recovery (Gwirstsman et al 1989a; Treasure et al 1985; Kennedy et al 1991). The most

substantial explanations for the hypercortisolemia are the impaired cortisol metabolism and CRF regulation. Besides malnutrition (Malozowski et al 1990), excessive exercise (Luger et al 1987), and stress are other conditions present in AN that are also linked to hypercortisolemia.

Insulin

Insulin is a protein hormone secreted in the pancreas by the islets of Langerhans and is released in response to elevated levels of blood glucose. Insulin acts to decrease blood glucose by increasing the cellular uptake and utilisation of glucose; it regulates the use and storage of body fuel molecules such as glucose, amino acids, fatty acids, and ketone bodies. Partial or total insulin deficiency results in diabetes mellitus.

Insulin administration induces LDL receptor expression, explaining the hypocholesterolemic effect of this hormone (Wade et al 1988). Insulin resistance and hyperinsulinemia increase the synthesis of hepatic VLDL and triglycerides and decrease HDL blood levels (Williams 1997). This lipid profile is linked to the increased risk of cardiovascular disease. However, insulin resistance can also result from increased mobilisation of non-esterified fatty acids from tissues as in cancer cachexia and starvation. There is no evidence that short-term periods of insulin resistance induced by such processes increase risk of cardiovascular disease.

Low fasting levels of glucose (<3.5 mmol/l) have been reported in a majority of the studies in AN (Schreiber et al 1991; Zuniga-Guajardo et al 1986). But symptomatic hypoglycemia rarely occurs. Hypoglycemic coma may take place in severely emaciated patients and may be a factor in the aetiology of sudden death. Recently, Wei and colleagues (2000) in a study of 40,069 subjects found a U-shaped relationship between fasting glucose levels and mortality. After adjustment for confounding variables had been made, those subjects with fasting glucose levels <70mg/dl (3.5 mmol/l) were found to have a 3-fold increased risk of cardiovascular mortality compared with the risk of subjects with fasting glucose levels from 80 to 109 mg/dl (4-5.5 mmol/l).

Besides hypoglycemia, patients with AN present similar abnormal glucose tolerance test (GTT) curves and sustained insulin response to those individuals (healthy or obese) suffering from starvation (Hales and Randle 1963). Impaired GTT (Crisp et at 1967; Silverman 1983) and normal GTT (Silverman 1977; Casper et al 1988a) have been described. Generally, blood glucose levels return to normal when patients have recovered; however impaired GTT has been reported to persist (Casper et al 1988b; Crisp 1965). Data on insulin sensitivity in AN is inconsistent. There are some reports of increased insulin resistance (Crisp 1965; Franssila-Kallunki et al 1991) as glucose disposal is reduced after GTT, while others have proposed an increased sensitivity to insulin in AN (Zuniga-Guajardo et al 1986; Wachslight-Rodbard et al 1979). Increased insulin receptors in erythrocytes in patients with AN (Wachslight-Rodbard et al 1979), which may explain the decreased plasma insulin and clearance of insulin after GTT, may be adaptive changes to avoid hypoglyceamia in response to low energy intake (Zuniga-Guajardo et al 1986).

Oestrogens

Oestrogens refer to a group of steroid hormones secreted by the gonadal glands, with 17 β-estradiol the most potent oestrogenic hormone. Oestrogens control sexual development in females, including development of sexual characteristics and maintenance of the menstrual cycle and reproduction. Pre-menopausal women are usually protected from blood lipid elevation by oestrogens. The use of hormone replacement therapy (containing oestrogen) has been associated with reduction in cardiovascular risk by between 40 and 60% (Stampfer and Colditz 1991). Oestrogen was used in the treatment of type II hyperlipoproteinemia in a group of postmenopausal women with a mean initial cholesterol value of 8.7 mmol/l. After six months of treatment with estradiol, cholesterol levels were reduced by 18% and HDL increased by 30% (Tikkanen et al 1978). HDL cholesterol levels in the population are higher in women than men and it is probable that this effect accounts for at least some of the cardiovascular advantages observed in young women (Kannel and Brand 1985). In addition, in animal experiments, Kovanen and colleagues (1979) demonstrated that the actions of oestrogen on lipids were: to lower blood cholesterol concentration, to

increase HDL by raising the number of LDL receptors and to decrease the HDL receptor affinity. Oestrogens have also beneficial effects on the vascular function (for example, inhibiting vasocontriction and migration of vascular smooth cells), the homeostasis (decreasing fibrinogen levels) and protecting LDL from oxidation.

Patients with eating disorders are amenorrheic as a result of low Follicle Stimulating Hormone (FSH) and Luteinising Hormone (LH) release. The DSMIV (1994) definition of AN explains that all patients with AN lose their menstrual bleeding for at least three consecutive months at some point in the course of their illness. Amenorrhea is a mere consequence of weight loss and it can be expected to develop in 80% women who have lost 12% of their ideal weight (approx. 8kg) (Pirke et al 1987). Amenorrhea, weight loss and psychiatric disturbances appear to occur together and, generally, the menstrual cycle normalises with weight gain and psychological treatment. Weight loss related amenorrhea is an adaptive form of fertility control in response to energy deficiency.

AN has been compared with a menopausic state in the light of the low oestrogen levels (Newman and Halmi 1988). The cessation of the ovarian function in menopausal women may predispose them to cardiovascular disease, since there is an increase in cardiovascular mortality in women following the menopause. The increased cardiovascular risk, due to oestrogen deficiency, may also be the case of patients with AN.

Depression and stress

Blood lipids can be modified by the emotional state of the patient. Depression has been connected with high (Nakao et al 2001) and low levels of total cholesterol (Brown et al 1994). Clinically, eating disorders and depression may be difficult to distinguish from one another because of their shared signs and symptoms, familial tendencies and response to medication. Depressed mood, pessimism, unwarranted worrying, constant misery and diminished capacity for enjoyment are commonly seen in patients with AN. Also, starvation itself produces cognitive, affective and social changes resembling major depression (Keys et al 1950). Therefore, the psychological

profile of patients with AN, characterised by a depressive mood, may contribute to the hypercholesterolemia.

Friedmann and colleagues (1959 and 1964) associated a particular behaviour pattern with cardiac risk. Cardiovascular disease appeared to be several times more frequent among 'type A' individuals than among those with the opposite characteristics, labelled 'type B'. The 'coronary prone behaviour' characteristic of 'type A' individuals included competitiveness, aggressiveness, haste, anxiety, restlessness, sense of urgency, inability to relax, high levels of drive and achievement. Elevated cholesterol concentrations have been found in 'type A' individuals (Friedmann and Rosenman 1959), in students a few hours before taking final examinations (Dreyfuss and Czaczkes 1959), and after experimental stress (mental task) (Muldoon et al 1992). Stress and cortisol excess are characteristics of AN which may also be linked to elevated blood lipids levels.

Weight loss

Raised blood cholesterol during weight loss has been reported. Young and colleagues (1953) studied six overweight women undertaking a weight reducing diet. By the end of the weight reducing period total cholesterol levels had increased in all subjects and in two of the subjects exceeded the normal values. Likewise, Savendahl and Underwood (1999) fasted (no food intake) eight women and six men for 7 days and observed an increase in serum cholesterol, LDL and apo B after the experiment. However, decreased blood total and LDL cholesterol and decreased HDL with weight loss have also been reported (Stevenson et al 1988) and this discrepancy may be explained by different characteristics of the subjects, degree of obesity, type of obesity (for example, accumulation of abdominal visceral fat), amount of weight loss and experimental study designs.

Low blood cholesterol levels have been found in subjects with protein-calorie malnutrition (Smith et al 1975) and in starved healthy volunteers (Keys et al 1950). However, in contrast to what would logically be expected, to find similar results in patients with AN, the majority of the studies have reported high cholesterol. The

elevated levels of cholesterol in AN are likely to be the result of delayed catabolism, as a mechanism to save energy given a lengthy decrease in the food intake and this does not seem to happen in starvation.

Lipid abnormalities have also been reported in other wasting illnesses, such as HIV (Manfredi 2000) and cancer (Vlassara et al 1986). In HIV, abnormalities in cholesterol levels are associated with a type of treatment including highly active anti-retroviral medication, while in cancer there is only an elevation of triglyceride levels, but not cholesterol, due to a low activity of the enzyme lipoprotein lipase (LPL). Therefore, the lipid anomalies in patients with cancer, HIV and AN have different aetiologies.

Dietary fats

Saturated fatty acids (SFA)

Fatty acids with chain lengths up to and including 10 carbon atoms (short and medium chain fatty acids) do not raise plasma cholesterol because they are absorbed directly into the blood and quickly metabolised in the liver. Lauric acid (12:0), myristic acid (14:0) and palmitic acid (16:0) are the 3-cholesterol-raising fatty acids, mainly present in meat, whole fat dairy products and tropical oils (coconut and palm kernel oil). Possible mechanisms include the inhibition of the removal of LDL from plasma by interfering with LDL receptors in the liver and the stimulation of LDL synthesis. Epidemiological studies showed that those countries with higher SFA intake tend to have the highest rates of coronary heart disease (Shaper 1988). The Nurse Health Study (Hu et al 1997) demonstrated that those women (34 to 59 years old) with the highest SFA intake were more likely to suffer from cardiovascular disease when they were investigated 14 years later.

Polyunsaturated fatty acids (PUFA)

PUFA can be divided into essential and non-essential fatty acids. The essential fatty acids (EFA) can be sub-grouped into either the *n*-6 series or the n-3 series. They

are essential because the body cannot synthesise these fatty acids *de novo* and their absence from the diet may cause a deficiency disease. There is a requirement for both linoleic (18: 2 *n*-6) and linolenic (18: 3 *n*-3) acids as a result of a lack of desaturase enzymes, which place double bonds in the *n*-6 and *n*-3 position. Linoleic acid is present in vegetable oils, such as corn oil and sunflower oil and linolenic acid in leafy plants and seeds. Both fatty acids undergo desaturation and chain elongation changes to give longer chain derivatives. *N*-3 derivatives include eicosapentaenoic (EPA) and docosahexaenoic acids (DHA), which are found in fish oils, and the *n*-6 derivatives include arachidonic acid found in meat and eggs.

N-6 fatty acids decrease blood TC, LDL and HDL. There is competitive inhibition between fatty acids, with *n*-3 fatty acids suppressing *n*-6 metabolism and n-6 fatty acids suppressing n-3 metabolism. Due to this fact it is important to maintain the balance of *n*-3 to *n*-6; the suggested ratio of linoleic to α-linolenic acid is 10:1 (FAO 1993). Otherwise, the decrease in *n-6* fatty acids may increase blood cholesterol levels.

Oleic acid is a non-essential monounsaturated fatty acid, mainly present in olive oil, which raises HDL values modestly. If there were a low EFA intake, mead acid synthesis from the elongation and desaturation of oleic acid would be enhanced, since $\Delta 6$ desaturase activity increases as a result of the lack of the other enzyme substrates: linoleic and linolenic acids. The ratio of mead acid to arachidonic acid greater than 0.2 is an indicator of EFA deficiency (Sanders 1988). Essential fatty acid deficiency is rarely observed in the free-living population and it has only been described in subjects undertaking parenteral nutrition. EFA deficiency has been linked to abnormalities in the cardiac function, homeostasis, immunity and reproductive system (Sanders 1988). Appendix 1.4 shows the major roles of EFA in the organism and the metabolism of fatty acids.

The restricted energy and fat intake in AN (Van Binsbergen et al 1988 and Drewnoski et al 1988) predisposes patients to incur nutritional deficiencies. Low EFA concentrations (linoleic and linolenic acids) are likely to take place in patients with AN, which may follow by increased blood lipid levels.

Antioxidant lipid-soluble vitamins

Lipid-soluble vitamins carotene and vitamin E have been shown to help prevent cardiovascular disease due to their strong antioxidant properties (Riermersma et al 1991 and Olson 1996). Vitamins E and carotene exert antioxidant activity through impeding LDL from oxidation by breaking the free radical chain reactions. PUFA contained in LDL particles are particularly vulnerable to reactive oxygen species because they have unstable double bonds in their structure. Uncontrolled lipid peroxidation can lead to cellular damage and be a trigger reaction for the formation and rupture of atheromatous plaque.

An inverse relationship was found between dietary intake of Vitamin E, carotene and Vitamin C, and mortality from cardiovascular disease (Knekt et al 1994). The Nurses' Health Study (Stampfer et al 1993) also found a positive association between Vitamin E intake (either in food or supplements) and a low risk of coronary heart disease. Epidemiological studies have shown that individuals who report consuming diets rich in carotene have lower rates of heart disease (Olson 1996) than the general population. Supplementation studies are controversial; in patients at high risk of cardiovascular disease this showed no benefit in terms of decreased risk of cardiovascular disease endpoints (Collins et al 2002). Conversely, β-carotene supplementation has shown to improve oxidation damage significantly in pre-menopausal women in a metabolic unit study (Dixon et al 1998), but not in male smokers (Cancer Prevention Group 1994).

Carotene

Carotene is present in yellow-orange fruits and vegetables. On absorption from the diet, carotene can be split in the middle by the B-carotene 15, 15-dioxygenase, which produces two molecules of retinaldehyde, and retinaldehyde reducatase, which converts it to retinal. Provitamin A carotenoids include: α, β and γ carotene, (β carotene is the most active form) and β cryptoxanthine (3-hydroxy β carotene). Lycopene and lutein (3, 3,'dihydroxy α carotene) cannot be converted into retinol and

therefore have no provitamin A activity. Six times the amount of β carotene and twelve times the amount of other provitamin A carotenoids (α and γ carotene and β cryptoxanthine) are required to equal the action of retinol. About 10% of carotenoids can function as vitamin A precursors and this conversion is highly regulated. The efficiency of conversion of pro-vitamin A carotenoids can be affected by many factors, such as dietary lipids, protein and bile salts.

Vitamin A or retinol is involved in visual function, in the control of cell differentiation and turnover, and in the immune system. Dietary sources of retinol are animal products such as liver and fish oils, dairy products and eggs. Retinol in blood is transported by the retinol binding protein (RBP), which is a short life protein (7 hrs.). RBP blood concentration depends on the adequate production of pre-albumin (transthyretin). The excess of retinol in the body is stored in the liver, esterified with long chain fatty acids, which are used to maintain a steady concentration of retinol in the blood. Retinol deposits in the liver are said to be enough to provide retinol for two years to a subject on a retinol free diet. Only when the liver reserves of retinol are exhausted, the plasma retinol levels decrease. A blood retinol level below 20μg/dl is regarded as deficient.

Vitamin E

Vitamin E includes α, β, γ and δ tocopherol (γ tocopherol is the most active form). Dietary sources of vitamin E are vegetable oils and margarine. After absorption, tocopherol is transported by lipoproteins through the blood. Blood levels of tocopherol depend on the dietary input of the vitamin, the concentrations of LDL, the rate of removal from the blood to the tissues, the levels of selenium, retinol, and sulphur amino acids. Vitamin E maintains the intracellular membrane integrity protecting constitutional fatty acids from oxidation. Vitamin E requirement increases when PUFA in the diet are high (Sanders 1993) as protects LDL from oxidation.

Vitamin deficiencies are more likely to occur in protein-calorie malnutrition. Decreased dietary intake and low blood concentrations of antioxidant vitamins have been described in AN. Decreased blood tocopherol concentrations have been

observed (Moyano et al 1999; Vaisman et al 1992). Levels of other antioxidant vitamins such as vitamin C (Christopher et al 2000) and of the antioxidant marker superoxide dismutase (Moyano et al 1999) have also been reported to be low. All these abnormalities tend to revert to desirable values during re-feeding.

Studies assessing lipid-soluble vitamin levels in AN were selected in a journal search performed in PUBMED (Table 1.4). The entry data were unlimited in years and one of the inclusion criteria was that the papers were in English. Key words were 'vitamins and AN'. Relevant references from these articles and from the review articles were also included. Those papers that only discussed BN, those whose sample sizes were smaller than five subjects, or review papers, were excluded. Searches were carried out from March 1999 to February 2003.

Table 1.4. Studies reporting lipid-soluble vitamin concentrations (Mean and N) in patients with AN

Author and year of study	**Retinol (μg/dl)**		**α-Tocopherol (μmol/l)**		**β-Carotene (μg/dl)**	
	Patients	**Controls**	**Patients**	**Controls**	**Patients**	**Controls**
Robboy et al (1974)	15.27 (8)	8.95 (10)	-	-	89.9 (8)	23.1 (10)
Casper et al (1980)	51 (22)	50 (33)	-	-	214 (27)	13.0 (32)
Curran-Celentano et al (1985)	63.1 (21)	67.8 (5)	-	-	77.7 (21)	16.8 (5)
Van Binsberger et al (1988)	45.5 (20)	33.8 (10)	25.3 (20)	22.1 (10)	123.5 (20)	114.9 (10)
Mira et al (1989)	57.3 (60)	48.7 (23)	19.5 (60)	16.5 (23)	-	-
Vaisman et al (1992)	39.8 (7)	46.5 (7)	13.9 (7)	22.7 (7)	-	-
Rock and Swendseid (1993)	-	-	-	-	88.9 (9)	47.1 (18)
Lagan and Farrell (1985)	58.7 (15)	53.7 (11)	28.1 (15)	23.4 (11)	-	-
Rock and Vasantharajan (1995)	54.4 (13)	N/R	23.7 (13)	N/R	-	-
Moyano et al (1999)	-	-	22.3 (82)	N/R	-	-
Boland et al (2001)					237 (101)	160 (95)
Total	**42.8 (166)**	**38.7 (99)**	**22.1 (197)**	**21.2 (51)**	**138.5 (186)***	**62.4 (170)**

* p: 0.018

N/R: not reported

Table 1.4 demonstrates that previous studies show conflicting results regarding retinol and tocopherol levels and that hypercarotenemia is a significant problem in the disorder. The main limitations of these studies are that vitamin values were not adjusted for TC and the different inclusion criteria used for the subjects.

Homocysteine

Homocysteine is a sulphur-containing amino acid, implicated in the metabolism of methionine, which is the principal methylating substance in the organism. Methionine is necessary for nucleic acid and protein synthesis, as well as for the integrity and correct function of cell membranes. The amount of homocysteine in extracellular compartments (plasma and urine) expresses the balance between intracellular homocysteine production and utilisation. Intracellular homocysteine has a short half-life and is exported into the extracellular media, especially when its production is increased. About 70% of homocysteine in plasma is bound to plasma protein (albumin), and the rest exists as a combination of mixed disulphides. A review of homocysteine metabolism is shown in Appendix 1.3.

Homocystinuria is a congenital inborn error of metabolism, which is caused by a homozygous defect in an enzyme: cystathionine B-synthase. The failure in the activity of this enzyme leads to an important increment of plasma homocysteine. McCully (1969) studying children with homocystinuria, observed that they frequently suffered from vascular disease at an early age and proposed that homocysteine was a cause of atheriosclerosis in humans. Increased levels of plasma homocysteine have subsequently been postulated as a strong risk factor for cardiovascular disease.

Nygard and collaborators (1997) found a strong graded relationship between plasma homocysteine levels and overall mortality in patients with heart diseases. Israelsson and co-workers (1988) detected increased levels of plasma homocysteine in patients with myocardial infarction before the age of 55, compared with controls. These patients presented a low incidence of conventional risk factors like smoking, hypertension and serum cholesterol. Furthermore, endothelial damage, platelet

sequestration, and venous thrombosis were observed in rats given a homocysteine injection (Hladovec 1979).

Hyperhomocysteinemia has been postulated as causing vascular damage by several means, including the impairment of flow-mediated vasodilatation and the injury of vessel wall (Lentz et al 1998). Homocysteine can stimulate smooth cells to grow abnormally in the arterial endothelial lining and these may become sites of cholesterol plaque formation; cholesterol and other lipids may then be a direct consequence of abnormal smooth cell growth (Beier 1984). There are also interactions between homocysteine and plasma lipids. Homocysteine can oxidise LDL in the presence of redox metals. When homocysteine is oxidised to homocystine, hydrogen peroxide is produced and the generation of oxygen radicals may modify lipid structures. Modified LDL may not be recognised by the LDL receptor but, rather, interacts with the scavenger receptor. This may contribute to the accumulation of cholesterol esters in foam cells, which are often found in early atherosclerotic lesions (Parthasarathy 1987).

Blood homocysteine concentration is elevated in deficiencies of vitamin B6, B12 and folate and is decreased by supplementation with these vitamins (Ubbink et al 1993). However, the optimum reduction with folic acid is only achieved with an intake of 800μg/d, which is considerably greater than the RNI for the vitamin (Wald et al 2002). However, Doshi and colleagues (2001) suggest that although folic acid supplementation lowers plasma homocysteine concentration and improves endothelial function these latter two processes may not be causally linked.

Homocysteine as a risk factor for cardiovascular disease has been criticised, especially by Brattstrom and Wilcker (2000). The arguments were that: 1. Not all the prospective studies have proved an association between high homocysteine values and cardiovascular disease. 2. Other confounding risk factors, such as smoking and high blood pressure, besides homocysteine may be responsible for the cardiovascular disease. 3. The gene for the folate-dependant enzyme MTHF reductase, which is associated with hyperhomocysteinemia, is not linked to future cardiovascular disease. 4. Hyperhomocysteinemia affects veins more than arteries. Ueland and colleagues

(2000), however, defended homocysteine as an independent risk factor and showed that the vast majority of the existing studies demonstrated a connection between high homocysteine values and cardiovascular disease, and that the evidence on the MTHF reductase gene has little statistical power.

Patients with AN suffer from malnutrition, altered eating patterns, vitamin deficiencies, oestrogen abnormalities and depression. All these conditions may alter homocysteine metabolism. Homocysteine has been scarcely studied in AN. Moyano and co-workers (1998) were one of the few researchers measuring homocysteine, vitamin B12 and folate levels in a group of teenager girls (n=43) with AN. They found high levels of plasma homocysteine (10.35 μmol/l), compared with reference values (7.35 μmol/l), but plasma vitamin concentrations were inside reference ranges. Homocysteine levels returned to normal after nutritional rehabilitation.

Philipp and colleagues (1988) observed that the intake of vitamins B12, B6 and folate was clearly reduced in a group of patients with eating disorders. Van Binsburgen and co-workers (1988b) concluded that in AN there is a depletion of vitamin B12 storage and that abnormal blood concentrations may reflect an increased catabolic state. However, it takes 3-4 years for stores of vitamin B12 to be depleted. Consequently, short-term food restriction is unlikely to result in vitamin B12 deficiency. Besides, reduced blood concentrations of folate (Mant and Faragher 1972) and vitamin B6 (Rock et al 1987) have been observed. Deficiency of these vitamins suggests that homocysteine metabolism in AN may be impaired.

Fibrinogen

Fibrinogen is a blood soluble protein that originates in the liver and is one of the blood-clotting factors. High fibrinogen levels contribute to platelet aggregation, increasing blood viscosity and influencing the amount of fibrin formed when coagulation is initiated. When blood vessels are injured, fibrinogen is converted to an insoluble fibrin matrix that acts as the scaffold for blood clot formation. During wound healing, the fibrin scaffold is dissolved and the clot is removed. Most acute myocardial infarctions are now known to be due to acute thrombosis, or the sudden

formation of a blood clot at the site of a ruptured atherosclerotic plaque. Antiocoagulants such as Warfarin is used for the preventive treatment of cardiovascular disease.

Prospective studies in healthy subjects have shown that a single fibrinogen measurement predicts fatal and non-fatal cardiovascular events as much as 16 years later (Meade et al 1993). The Gothenburg study (Wilhelmsen et al 1984) first demonstrated that the incidences of stroke and myocardial infarction were greater in the men who 13.5 years earlier had the highest fibrinogen levels. The Northwick Park Heart Study (Meade et al 1986) was designed to investigate the thrombogenic components of cardiovascular disease. Over 1500 white men aged 40-64 years, who were free of heart disease, were examined and then followed up over a period of 10 years. In this study the range of fibrinogen concentrations was divided into tertiles and cardiovascular death expressed by thirds of overall distribution, about 60% of cardiovascular deaths occurred in the highest tertile for fibrinogen concentrations. In the Framingham Study a 12-year follow-up showed that the risk for developing cardiovascular disease was associated with the antecedent fibrinogen value higher than 1.3 to 7 g/l (126 to 696 mg/dl) (Kannel et al 1987).

Fibrinogen has been shown to be an independent and valid indicator for future cardiovascular disease in young adults (Folsom et al 1993). Several studies have shown that elevated fibrinogen concentrations are related to increased age, heredity, tobacco smoking, obesity, diabetes mellitus, stress, high blood pressure, high cholesterol concentrations, low levels of exercise and alcohol abuse (Meade et al 1986; Imenson et al 1989; Folson 1991; Stec et al 2000).

Fibrinogen in AN has been poorly studied. However, fibrinogen tends to cluster some of the main risk factors for cardiovascular disease, which patients with AN often manifest.

1.2.2. Osteoporosis

1.2.2.1. Definition and epidemiology

Osteoporosis is a progressive condition in which the bone matrix and bone minerals are lost with a consequent loss of bone mass and bone fragility. Osteoporosis is defined as a value of BMD 2.5 SD or more below the mean for young adults (WHO 1994). Normally, all people gain bone during the growing period, the peak bone mass being attained at 18-20 years and it descends when people age. Up to 80% of a subject's BMD can be attributed to genetic factors (Pocock et al 1987). However, an individual who does not reach an optimal bone mass during childhood and adolescence may develop osteoporosis later in life. Preventive campaigns have, therefore, been focused on increasing the peak bone mass formed during adolescence and decreasing the subsequent bone loss. If bone loss is quick, fractures are expected particularly in the forearm (Colles fracture), the spine and the hips. All these areas contain a high tubercular bone, which has high metabolic activity.

Osteoporosis is a major world-wide public health concern. Epidemiological studies showed that 45% of white women aged 50 or more years have reduced BMD (Melton et al 1992). Mosquera and colleagues (1998) showed that the incidence of femur fracture in Argentinean women (>50years) from Buenos Aires was 259,6 per 100,000 inhabitants which is high compared with 114,9 in England (Oxford-Dundee) in women older than 35 years (Melton and Riggs 1987). The projection is that osteoporosis will grow world-wide, but the greatest increase will be in Latin-American countries.

Osteopenia (BMD between 1 and 2 SD below maximum expected for sex-matched healthy subjects) is a main concern in AN, coupled with the fact that the age of onset of AN is mostly a period where peak bone mass is often achieved. The impact of treatment for AN on bone health is controversial. Rigotti and colleagues (1991) observed that BMD remains low in spite of weight gain and return to menstruation, and the risk of fracture was 7 times that of healthy controls. Conversely,

Hotta and colleagues (1998) and Bachard and colleagues (1990) demonstrated a significant increase in BMD after weigh gain in patients with AN.

1.2.2.2. Risk factors for osteoporosis

Decreased BMD and increased risk of fractures are common features of AN (Grinspoon et al 1999). A negative net balance between bone resorption and bone formation leads patients to bone loss and osteoporosis. Some of the factors known to increase bone loss in AN include: a prolonged duration of the illness, low BMI, hypercortisolemia (chronic stress), hypoestrogenemia (amenorrhoea), strenuous exercise and inadequate calcium intake.

Duration of the illness

Low BMD is positively associated with duration of emaciation (Treasure et al 1987). The earlier the onset and the longer the duration of illness the greater the loss of bone. The deficit of bone mineralisation that would have happened during adolescence is irreversible.

Low BMI

Obese women are less likely to develop osteoporosis than thin women. Obesity protects against bone loss in menopause by increasing the amount of oestrogens because there is more adipose tissue necessary to metabolise oestrogens. Previous studies have shown that anorexic patients, with low body weight and low BMI have decreased BMD (Treasure et al 1987; Bachard et al 1990).

Hypercortisolemia

Cortisol excess is associated with both impaired bone formation and increased bone resorption (Newman and Halmi 1989). Low BMD in AN has been explained by the high levels of cortisol, which are secondary to stress (Biller et al 1989).

Hypo-oestrogenemia

Oestrogen deficiency after the menopause is the main cause of bone loss in women. Similarly, the hypooestradiol state in AN leads to an increase bone turnover (cycles of bone resorption and formation) and to a high bone loss rate with a consequent reduction in BMD. However, the duration and degree of oestrogen deficiency and amenorrhea in AN determine bone loss (Kiriike et al 1992; Treasure et al 1987).

Strenuous exercise

Excessive exercise, which is a common feature of AN, causes bone loss. Women with exercise induced amenorrhea, such as professional dancers and athletes, have decreased BMD (Frusztajer et al 1990). On the other hand, moderate exercise (3-4 hours of weight-bearing exercise/week such as walking) is protective for the health of the bones.

Low Calcium intake

An optimum calcium intake promotes bone mineralisation during adolescence and regulates the rate of bone loss through age. The diet of patients with AN is often calcium deficient, since many avoid dairy products, and this is therefore a factor contributing to decreased BMD. However, calcium supplementation in AN does not correct bone loss or increase bone density (Rigotti et al 1991)

This section has been covered briefly because the study of risk factors for cardiovascular disease was the main emphasis of this thesis. However, osteopenia and the risk of osteoporosis are relevant problems in young women and in patients with AN and, therefore, these are addressed in Chapters 2 and 5.

Chapter 2 - Risk factors for osteoporosis and cardiovascular disease in adolescent women

(Study 1)

Chapter 2- Risk factors for cardiovascular disease and osteoporosis in adolescent women

2.1. Introduction

Adolescence is the time of life when behavioural patterns, which will be carried into adulthood, are formed. Teenagers are first exposed to many risk factors for chronic diseases such as tobacco smoking, alcohol intake, dieting and other unhealthy behavioural patterns to lose weight.

Evidence suggests that some school age females exhibit a similar pattern of behaviour to patients with AN. Young girls are often preoccupied with their weight and body shape (Hill and Rogers 1992). Dissatisfaction with appearance frequently leads to dieting, which places them at risk of nutritional deficiencies, eating disorders and other long-term health problems. Vomiting, laxative, diuretics and tobacco use are forms of weight control often adopted by adolescents (Neumark-Sztainer et al 1999).

Tobacco smoking, one of the most significant risk factors for future chronic diseases, is a prevalent practice in adolescents (Bolling 1993). Smoking and body weight have been linked to social class (Halek et al 1993), and to being brought up in a single parent family (Goddard 1990). Health and social status have been associated, with a trend of increasing mortality in lower social levels (Smith and Baghurst 1992). These differences may be a consequence of diverse lifestyles among the social groups, including dietary, smoking, exercise and alcohol patterns. This suggests that risk behaviour likely to incur in future disease depends on the socio-economic level.

Schoolgirls have a potential increased vulnerability to eating disorders, osteoporosis and cardiovascular disease. This field has not been researched in Argentina, where there is a socio-cultural drive towards thinness (Zuckerfeld et al 1988; Bello 1995; Garcia 1997; Meehan and Katzman 2001), a high incidence of

osteoporosis (Mosquera et al 1998) and cardiovascular disease (Hauger-Kevene and Balossi 1987).

This chapter aims to investigate attitudes and behavioural patterns related to chronic diseases in schoolgirls from different socio-economic backgrounds. The objectives of this study were to assess: 1. Food intake, 2. Anthropometric measurements, 3. Behavioural risk factors for chronic diseases (alcohol intake, tobacco use and exercise) and 4. The relationship between dieting to lose weight and body dissatisfaction in a group of Argentinean schoolgirls aged between 15 and 17 years.

The hypotheses to be tested were:

- That schoolgirls have patterns of behaviour (lack of exercise, tobacco smoking) and diet (low energy intake, high saturated/polyunsaturated fat ratio, and low calcium and fibre intake) incompatible with cardio and bone protective recommendations.

- That patterns of behaviour including lack of exercise, tobacco smoking and diet characteristics (such as low energy intake, high saturated/polyunsaturated fat ratio, and low calcium and fibre intake) are more prevalent in girls from state than from private schools.

- That tobacco smoking is more frequent in those girls living with a single parent and in those having a relative or close friend who smokes.

- That coffee and alcohol use, low meal frequency and tobacco smoking are associated with a low perception of health control and a lack of health concern when choosing what to eat.

- That dieting to lose weight and coffee and alcohol use are more frequent in those girls having at least one meal out of home.

- That skipping meals is the most frequent method to lose weight

- That patterns of the diet (coffee and alcohol use, vegetarianism, low meal frequency) and behaviour (tobacco smoking, dieting, use of laxatives, diuretics, slimming pills and vomiting) are positively associated with body dissatisfaction.

- That body dissatisfaction in schoolgirls is positively correlated with the percentage of body fat.

2.2. Methods

2.2.1 Design

This was a cross-sectional study. Schoolgirls were recruited from one private school and two state schools in Buenos Aires. Schoolgirls from the private school were from a higher socio-economic background than the girls from the state schools.

A 7-day food diary of estimated food intake assessed the diet of schoolgirls. Data from the diet (total energy intake, fibre, polyunsaturated fat, saturated fat, calcium intake and other nutrients) were compared with the WHO recommendations (1990) and the Dietary Reference Values (1991). A Lifestyle and Background Data Questionnaire assessed dietary patterns and behaviour connected to chronic diseases. The Body Rating Scale assessed body satisfaction. Anthropometry was used to calculate BMI and percentage of body fat.

2.2.2. Sample

The size of the sample was determined as a function of the number of subjects it would be possible to study in a limited time (three months) and of the number of schools that the Argentinean Ministry of Education allowed to be studied. The age group of the subjects was designed to be relatively similar to the estimate mean age of onset of AN (Herpertz-Dahlmann et al 1996; Vandereyck and Pierloot 1983).

Inclusion criteria

- Girls from 15 to 17 years old attending state and private secondary schools in Buenos Aires.

Exclusion criteria

- Male sex
- Pregnancy

2.2.3. Procedure

Selection of the schools

The Buenos Aires Sub-secretary of Education was asked to select two state schools from different socio-economic backgrounds to undertake this study. The schools called Emem 4 and Comercial 12 were selected based on the profession of the students' parents and the location of the school.

Many of the variables studied may differ according to the socio-economic background of the girls. Therefore, two private schools were also invited to take part in the research project for comparison with the state schools. These private schools were selected on the condition that they had the following characteristics:

1. High number of registered students. This is because, according to the information of the local government, there are more students attending private schools (97,566) than state schools (91,627) (Dirección General de Estadísticas y Censos, Buenos Aires 1999). Therefore, the sample size of the private school was aimed to be bigger than the state schools.

2. Payment of a monthly fees of U$ 600 or more, considering that a minimum salary was U$ 450.

3. Located in a wealthy neighbourhood.

4. Parents occupation to be mostly professional.

Approval to carry out the research project was granted by only one of the two schools invited (Ort school).

Other relevant data on socio-economic level is shown in a governmental survey (Dirección General de Estadísticas y Censos, 1991), which shows the percentage of inadequate housing (for example: lack of potable water, electricity, inappropriate building, etc.) by neighbourhood (Appendix 2.6). These data demonstrate that the two state schools located in a neighbourhood of Villa Lugano have a significantly higher percentage of inadequate houses (14.5%) compared with the upper-income school, located in the neighbourhood of Caballito (1.7%). Although the two state schools were selected in the same neighbourhood, the schoolgirls of one of these schools (Emem 4) live in areas of the neighbourhood with inadequate houses, such as shanty town houses, and the girls from the other school (Comercial 12) in areas with adequate houses.

Preparation with school authorities

An explanation of the purpose of the study and the procedure involved was given to the principals of the schools. This was followed by from two to four visits to discuss the study further. Dates and times to carry out the fieldwork were organised with the schools. The research project was introduced as a study of health and nutrition among young girls and was carried out in the Biology or Physical Activity classes. The parents and schoolgirls were informed of the study and signed a written consent form to agree to participate in it (Appendix 2.7). The schools were offered a final report of the results.

Preparation with schoolgirls and fieldworkers

Schoolgirls were approached in the classroom. The visit started with a brief introduction about the study and they were given the consent form to be signed by their parents. In the next meeting girls were encouraged to complete the Lifestyle and Background Data Questionnaire. They were assured that the questions did not represent a 'test' and that there were no correct or incorrect answers. They were asked

to be as honest as possible and to fill in the questionnaires without conferring. It was explained that the results were confidential and that the study was not connected to school marks. That day they were also given an explanation as to how to fill the 7-day food diary, which they had to complete and bring back the following week. A portion size guide and portion size models (for example a medium size glass, plate, cup, spoon, etc.) were shown that day.

Food diaries, the body rating scale, blood pressure and anthropometry were assessed in a subset of girls who agreed to participate. The following week the schoolgirls were brought individually from the classroom to a testing area within the school to have, in order of execution, the following tests:

- Blood pressure measured
- 7-day food diary checked
- The Body Rating Scale assessed
- Anthropometry taken. A trained student from Dietetics (University of Maimonedes) and a trained dietitian helped with anthropometry. They were trained before carrying out the study on a Pilot study to validate the 7-day food diary of estimated food intake and to train on anthropometry.

Description of the tests applied

Blood pressure

Systolic and diastolic blood pressure were recorded by a mercury sphygmomanometer with an appropriate cuff (Kosan, Japan). Diastolic blood pressure was measured at Korotkoff phase IV. The recommendations for blood pressure measurement (Beevers et al 2001) were followed. Measurements were made with subjects seated after 3 minutes of relaxation and the forearm supported at the heart level. The same trained observer performed the measurement in duplicate and the readings were averaged to reduce the bias. Factors such as stress, cuff too large, which may have biased the readings, were noted and the measurements thought to be

invalid were excluded. It was not possible to control the time of the day the measurement was made.

The blood pressure results were compared with the following two Argentinean studies. Palmero and Caeiro (1971) show systolic and diastolic blood pressure values of girls from 10 to 19 years (N= 296) from the city of Cordoba, and Echeverria and colleagues (1988) describe blood pressure data of girls from 15 to 24 years (N= 767) from the city of La Plata. These studies are the most contemporary reports on blood pressure in young Argentinean females.

Dietary investigations

A 7-day food diary was administered (Appendix 2.2). Food models were previously shown to the girls to standardise precise portion sizes. A guide of serving sizes was attached to the food diaries (Appendix 2.3). These were checked at collection to see that they were correctly completed and any doubts about the information were discussed with each girl. The 7-day food diary was selected for this study as it is recommended for the assessment of individual intake and because while estimating seven days the variation between weekend and weekday intake is attenuated. However, changes in eating habits may occur when subjects are under pressure to record food intake. This method has been validated against food frequency questionnaires and biomarkers (Brunner et al 2001). This method of assessing food intake is weak compared with the duplicate diet studies and the weighed recorded methods. However, the non-weighed 7-day food diary was chosen because it was thought to be suitable for the population studied, the time allotted for the study and the financial resources available.

A computer programme (Argentinean programme: DietPlan) was used to estimate energy content, macronutrient intake, saturated and polyunsaturated fat, calcium, fibre, iron, vitamin C, A and others. This computer programme uses the diet composition tables of the Argentinean Institute of Nutrition, Centro de Endocrinologia Experimental y Aplicada (CENEXA); the University of Luján (Buenos Aires) and the Centre of Infant Nutrition Studies (CESNI). Unfortunately the

programme did not allow the analysis of simple carbohydrates (extrinsic sugar). The food diary was validated against a food frequency questionnaire in a student sample. Fifteen students from the Maimonedes University (Buenos Aires) were given a food diary and a translated version of the food frequency questionnaire of the Nurses' Health Study Dietary Questionnaire (Willet 1998). The portion size guide and food life models were shown to the participants before filling in the questionnaires. Reported total energy intake was significantly associated (p: 0.022) between the two methods by the Pearson correlation test and the mean values were comparable.

The method of Black and co-workers (1991) and Goldberg and colleagues (1991) was used to assess the validity of the recorded diets. This validation supports the fact that energy intake (EI) cannot be less than basal metabolic rate (BMR). The Schofield and colleagues equation (1985) was applied to estimate BMR and the figure of 1.1 was selected as the cut-off point for an acceptable ratio of EI to BMR. Subjects with values below this point could be classified as under-reporters. Under-reporting in teenagers have been demonstrated (Bandini et al 2003) and the accuracy of the report declines with age. Among the possible causes of under-reporting are: peer-pressure to declare the food they consider to be healthy, dieting to lose weight, frequent eating out and therefore difficulty in estimating the ingredients, and normal adolescent irresponsible behaviour.

Body rating scale

Stunkard and colleagues (1983) originally devised the figure drawings. They consist of seven figures ranging from a very thin female to a very fat female (Appendix 5.4). The girls were asked the following questions:

- ***Self***: *Which picture looks most like you?*
- ***Ideal Self***: *Which picture shows the way you want to look?*

The seven figures were labelled as: 1. Extremely thin, 2. Very thin, 3. Thin, 4. Normal, 5. Overweight, 6. Very overweight and 7. Obese in order to allocate the girls current BMI with the figure of the scale chosen to represent their current and ideal size. Allowing schoolgirls to demonstrate what they perceive to be their current and

ideal body sizes permits calculations to be made of how closely they believe themselves to resemble their ideal. Eating disordered women report significantly greater discrepancies than non-eating disordered women (Lindholm and Wilson 1988).

Methodological concerns using silhouettes were identified as follows (Garner et al 1998):

1) *Restriction range*: most of the subjects select 3 or 4 out of 7 figures.

2) The ascending presentation order of the figures, producing high test-retest reliability because subjects will remember the figure they chose before.

3) *Scale coarseness*, because information is lost when subjects are required to limit responses to one of a finite number of figures.

The BRS was chosen for this study because it has acceptable retest reliability (Thompson and Altabe 1991) and it was specially designed for undergraduates, which is a close age group to the schoolgirls of this study. Stunkard figures have been found to be as valid as other reliable methods of assessing body size perception, such as video procedures and other silhouettes scales (Garner et al 1999). The use of the BRS was also convenient because it requires minimal equipment and financial resources, compared with camera procedures and adjustable light beams and mirrors, and it is a quick instrument to be included in the battery of tests performed. One of the limitations of this test is that it has only been validated in Caucasian populations (Mautner et al 2000) but not in minority ethnic groups. The level in which indigenous girls from Argentina could identify with these Western-look silhouettes is, therefore, limited.

Background data and life-style questionnaire

The aim of the self-filled life-style questionnaire was to assess background data and to identify lifestyle behaviour, which may make subjects prone to chronic diseases. The questionnaire was designed in English and translated into Spanish. A back translation, by double-checking with a native speaker the meaning of each question, was carried out. The questionnaire was piloted in a student population (University of Maimonedes) N=10 and some adjustments were made to obtain a clearer understanding of the questions (for example specifying the meaning of 'dieting' as changing food habits in order to lose weight).

The questionnaire took approximately 20 minutes to complete (Appendix 2.1). It measured relevant variables such as: tobacco smoking, meal frequency (low meal frequency was defined as having two or less meals per day), alcohol and coffee consumption, vegetarianism, meals taken out of home, adults living with the girls, relatives or close friends, smoking and exercise pattern. Meals were defined as the intake of an important amount of food which usually takes place sitting down at a table, and snacks defined as a smaller intake which usually takes place away from home.

Exercise is difficult to measure accurately and in this study it was decided to use a self-report approach. Self-report methods are ideal when studying a large group of individuals and serve to group individuals into broad activity categories. Questionnaires to assess exercise level are generally more practical and less time consuming than other methods (accelerometer, diary annotation, direct observation and calorimeter), although less accurate. The performance of exercise was rated with the question – how many times in the last 7 days have you taken any physical activity for long enough to breath harder?

Extra questions about dieting behaviour were added to this questionnaire: attempts to change body weight by slimming, use of slimming pills, laxatives, diuretics, vomiting and dieting to lose weight. Questions on attitudes and beliefs toward health were also added, these include: perception of health control (low

perception was rated when choosing the option 'disagree' or 'not sure' to the statement 'I am in charge of my health') and consideration of health when choosing what to eat (the option "never' was rated as lack of health concern). Questions relating to diet and behavioural patterns were selected from a questionnaire of the School Health Education/Britain (Balding 1999) and from the Minnesota Adolescent Health Study/USA (Neumark-Sztainer 1998), which are questionnaires already tested for validity and reliability, and it was assumed that this would apply to Argentinean teenagers.

Anthropometric measurements (Appendix 2.5 for description of measurements)

Weight (kg) and height (m) were measured to calculate BMI (kg/m^2) and skinfold thinness to assess total body fat. These anthropometric measurements were chosen because they provide health indicators, which can be compared with reference values, and are related to cardiovascular and anorexic risk. Waist/hip circumference and arm circumference were other measurements taken but as they were not relevant to this thesis were not included.

BMI data were used as a comparison tool, however, adolescents are still growing and therefore it would not be appropriate to utilise BMI as an indicator of healthy weight to height. The results of weight and height were compared with the Argentinean reference values of Lejarraga (1986), which are National references centiles of 1) weight to age and 2) height to age for girls and boys from birth to 19 years. There are no reference values for body fat percentage in the young Argentinean females. Therefore, the results of body fat calculated from skinfold thickness were compared with the study of Durnin and Womersley (1974) which shows percentage of fat in British girls from to 12 to 14 years. The comparison with this study has the limitation of different samples assessed and different, although close, age groups.

2.2.4. Statistics

Frequencies and descriptive statistics were produced to describe the sample. The Chi^2 test of association was used for testing relationships between two nominal variables (e.g. dietary restraint and body dissatisfaction). The Pearson correlation test was used to associate two independent variables (e.g. energy intake and percentage of body fat). The one-way ANOVA test was applied to test for differences among more than two independent discontinuous variables (e.g. carbohydrate intake in low, middle and upper-income schools). Any difference was regarded as statistically significant if $p< 0.05$.

2.2.5. Data collection and ethical considerations

The Data Protection Act (1984) for the information held in computers was followed. All the collected information was codified and put into the SPSS computer programme.

The data collection period for this study was planned from July to October 2001. Ethical approval was granted from King's College London (99/00-91) and the Buenos Aires Sub-secretary of Education.

Declaration

The author of this thesis designed the study, carried out the fieldwork and analysed the data.

2.3. Results

The demographic characteristics of the schoolgirls and information about anthropometry, food intake and lifestyle behavioural patterns related to chronic diseases will be discussed in this section. Information with regards to the girls' appreciation of health and prevalence of AN and BN in the schools will also be presented here.

2.3.1. Characteristics of the schoolgirls and anthropometric data

The sample comprised 342 girls aged between 15 to 17 years old from three secondary schools in Buenos Aires. The forty-six girls from the low-income state school (Emem 4) had in general unqualified or unemployed parents. A high number of these were immigrants from border countries such as Bolivia, Paraguay and Peru. The one hundred and twenty three girls from the middle class state school (Comercial 12) had parents who were mainly qualified employees or professional people. The one hundred and seventy-three girls who attended the private school (Ort) were from an essentially upper socio-economic background, having predominantly professional or self-employed parents. The private school was located in central Buenos Aires and the state schools in a Buenos Aires suburb. The low-income school was centred in a deprived area opposite a shantytown. Low-income students attended school in the evening from 6 to 11 p.m. Girls from the middle-income group attended school in the morning from 8 a.m. to 1 p.m. or in the afternoon from 1 to 6 p.m. Upper-income girls stayed at school a longer period, from 8 a.m. to 3 p.m. All these data were gathered by direct observation and from interviews with the principals of the schools where they were asked – 'From which neighbourhoods do the girls mainly come?' and – 'What do the girl's parents mostly do for living?'

A total of approximately 650 girls were informed about the study. However, since participation in the research project was voluntary, different levels of co-operation were obtained for each of the tests and only the girls who accepted to take part were included. This is shown in Figure 2.1.

Figure 2.1: Diagram of the participation rate in the different tests of the study by type of school

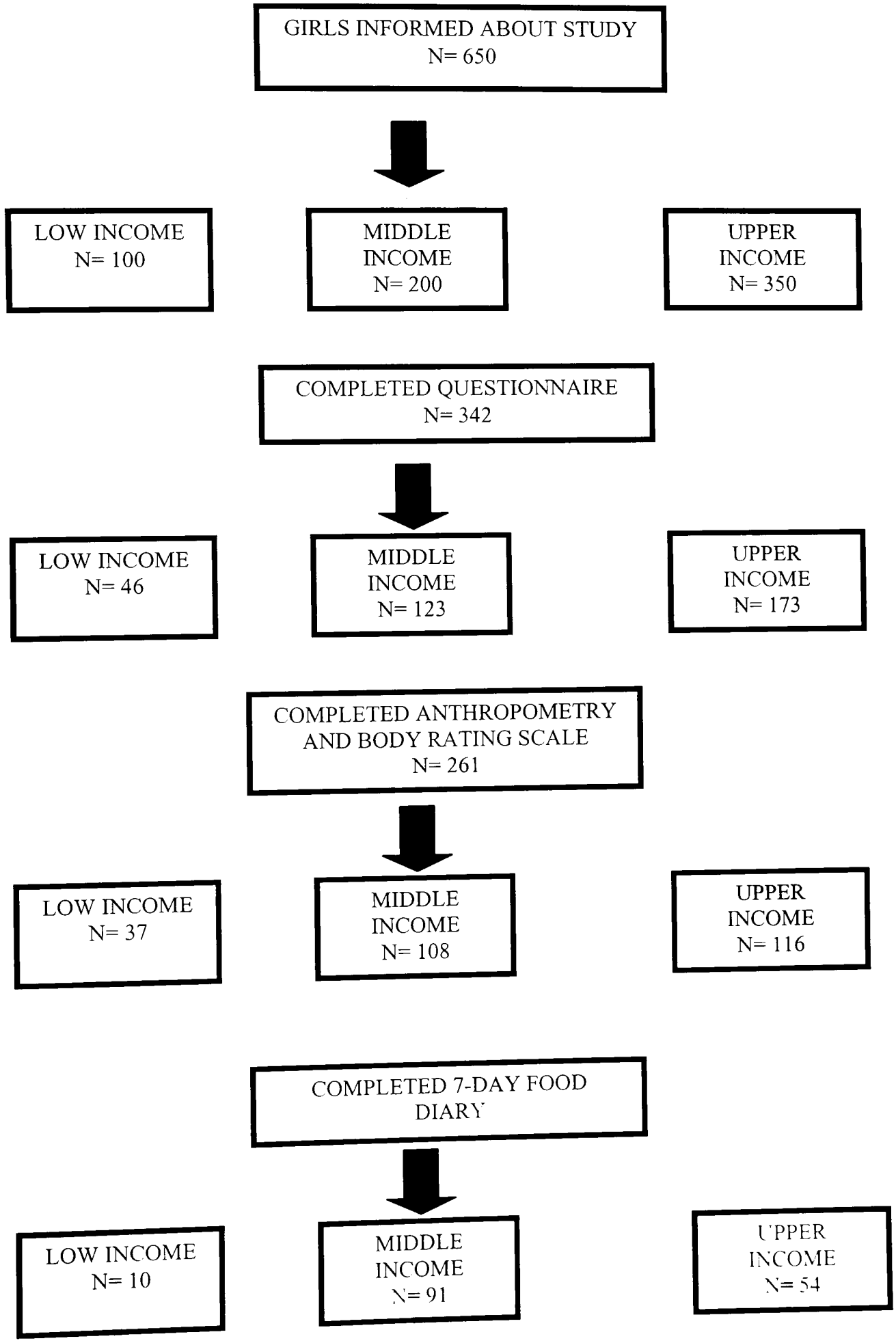


Table 2.1: Characteristics of schoolgirls by type of school

Variables	Low-income School				Middle-income School				Upper-income School				All schools together		
	N	Mean	SD	Range	N	Mean	SD	Range	N	Mean	SD	Range	N	Mean	SD
Age (years)	*46*	15.9	0.7	15-17	*123*	15.8	0.8	15.0-17.0	*173*	15.7	0.7	15.0-17.0	*342*	15.8	0.7
Height (m)	*37*	1.59	0.07	1.47-1.71	*108*	1.59	0.06	1.44-1.72	*116*	1.61	0.06	1.47-1.81	*261*	1.6	0.1
Weight (kg)	*37*	56.4	9.0	40.5-79.0	*108*	53.7	7.9	38.0-87.0-	*116*	54.2	7.1	42.5-79.5	*261*	54.3	7.7
BMI (kg/m^2) ∝ Ψ	*37*	22.0	3.4	17.7-30.8	*108*	21.1	3.0	15.6-33.6	*116*	21.0	2.3	15.6-28.4	*261*	21.0	2.8
Body fat from skin fold (%)♠Ψ	*37*	28.3	3.4	21.3-36.0	*108*	29.0	3.3	20.4-36.7	*116*	30.4	3.3	21.6-38.5	*261*	29.5	3.4

Anova test

Ψ $p < 0.05$ low-income school compared with upper-income school

∝ $p < 0.05$ low-income school compared with middle-income school

♠ $p < 0.05$ upper-income school compared with middle-income school

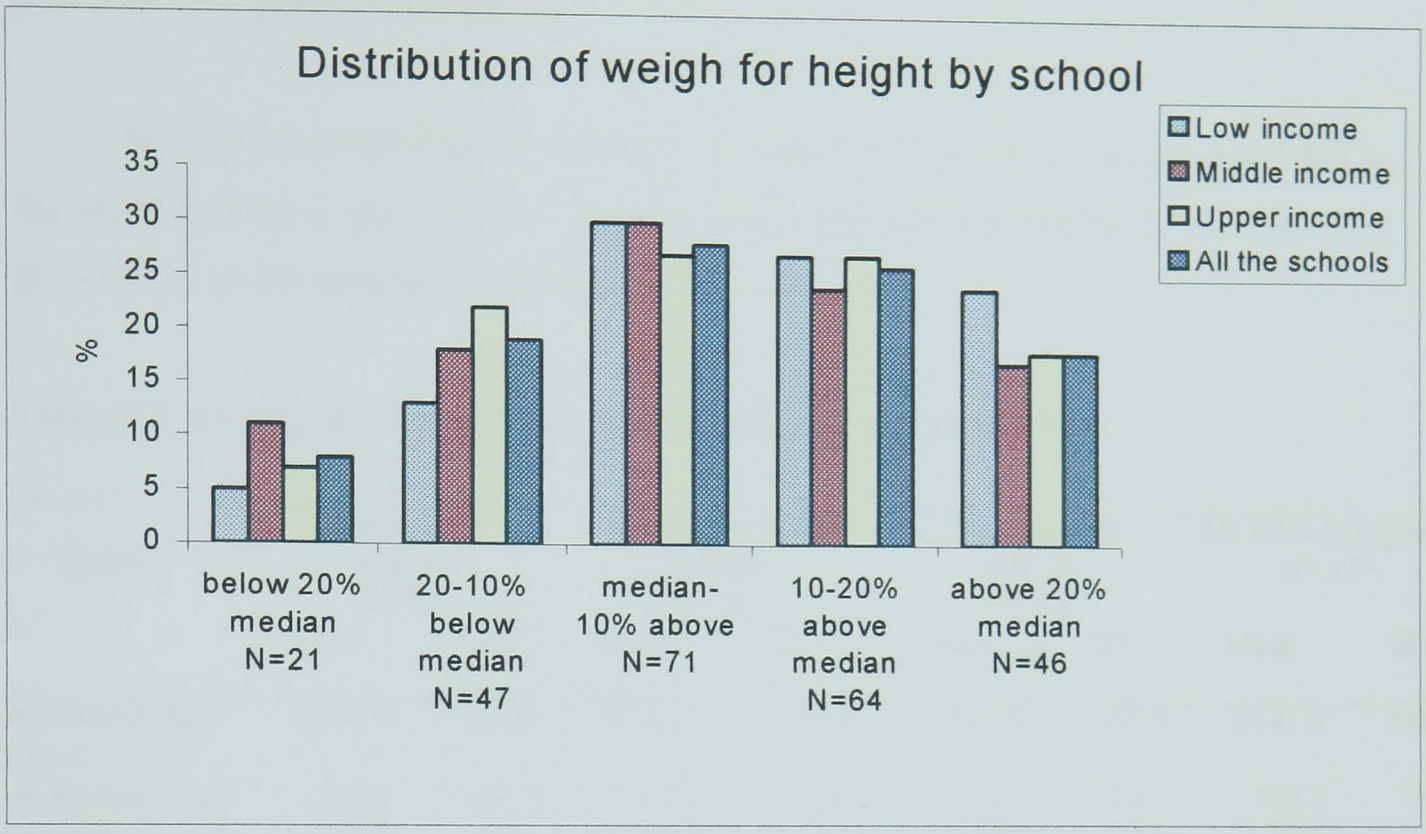
Anthropometric differences were noticed among the schools. Table 2.1 shows that BMI was significantly higher in the girls from the low-income school than from the middle and upper-income schools (p: 0.033 and 0.011 respectively). Argentinean reference values for girls aged 16 years at percentile 50 are 54.5 kg for weight and 1.60 m for height (Lejarraga et al 1986). The whole sample of girls had an almost identical value for body weight and height to these reference values. The mean weight of the low-income girls was slightly higher than reference values, although not significantly. This can be seen in Graph 2.1, which shows the distribution of weight for height in the sample compared with reference values centiles (Lejarraga et al 1986) and school. It is worth noticing the high number of girls from the low-income group with body weight for height above the median.

Percentage of body fat was higher in upper-income girls than low-income (p= 0.002) and middle-income (p= 0.004). Difference on the anthropometric data may be explained by a diversity of racial backgrounds of the girls, as most of the upper class schoolgirls were Caucasians while many of the low and middle socio-economic girls came from indigenous backgrounds. Body fat percentage calculated from skinfold thickness was 24% (SD 4.9) in a sample of British girls aged between 13 to 16 years (Durnin and Rahaman 1967) and 26% (SD 7.8) in a sample of girls between 16 to 19 years old (Durnin and Womersley 1974). These values are below the percentage of fat observed in this study, perhaps because the Durnin's studies included girls younger than the ones of this study.

Mean systolic (103.85 mmHg, SD 10.38) and diastolic (68.43 mmHg, SD 10.29) blood pressure differed significantly from the Argentinean studies of Palmero and Caeiro (1971) systolic: 115.1 mmgHg (SD 11.5) (p= 0.000) and diastolic: 70.1 mmHg (SD 9.05) (p= 0.010) and Echeverria and colleagues (1988) systolic: 118.8 mmgHg (SD 0.31) (p= 0.000) and diastolic: 73.3 mmHg (SD 0.24) (p= 0.000). Different age groups and methodologies may explain the discrepancy between the results of this thesis and these studies.

Age at menarche (mean 12.32 years, SD 1.20, N=333) was negatively associated with BMI (p= 0.000, r -0.288 Pearson correlation). Also age at menarche was significantly lower (p= 0.002, mean 12.1 years, SD 1.2 T-test) in girls who dieted compared with non-dieters (mean 12.5 years, SD 1.1). This suggests that heavier girls and those restricting their food intake had matured earlier.

Graph 2.1



With regard to the information on family set-up, most of the girls (68%) lived with both parents, and this percentage was higher in the upper-income school (84%). About 30% (N=14) of the low-income girls lived with the mother only. The mother was more likely to be the head of the family than the father. The girls living with both parents had a greater energy intake (mean 2200 kcal, SD 567 p= 0.043, T test) than those living with single parents (mean 1990 kcal, SD 541).

2.3.2.Food intake

2.3.2.1. Energy, macro and micro-nutrient intake

Incomplete food diaries (N=11) were discarded from the analysis of energy intake. 30% subjects had EI: BMR ratios below the cut-off point and they could be defined as underreported. However, these girls were not excluded from the dietary analysis because given the high number of girls currently on a diet, it may be possible that many of girls were intentionally restricting their food intake with a consequent

low reported energy intake and a distorted EI: BMR ratio. Of the girls with an EI: BMR ratio below the cut-off point (N=101), 27% reported dieting during the study year and 73% were not dieters.

It is to be noted that the dietary data from the low-income group is limited to the small number of girls (N=10) who agreed to complete the food diary and therefore the validity of the comparison is limited.

Table 2.2: Energy, macro and micronutrient intake by type of school

Intake	Low–income School N= 10		Middle-income school N= 91		Upper-income school N= 54		All schools together N= 155	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Total energy (EI) (kcal) ♠	2040.0	430.0	2231.0	592.0	2007.0	521.0	2141.0	566.0
Carbohydrate (g)	240.2	102.3	259.7	70.1	214.4	59.7	242.7	71.8
(% EI) ♠	(46)	(10)	(47)	(7)	(43)	(6)	(46)	(7)
Protein (g)	82.5	19.6	87.2	26.1	82.3	22.4	85.2	24.4
(% EI)	(16)	(3)	(16)	(3)	(16)	(2)	(16)	(3)
Fat (g)	83.5	12.9	94.8	30.7	90.9	26.9	92.7	28.6
(% EI)	(38)	(7)	(38)	(6)	(40)	(4)	(39)	(6)
Saturated fat (g)	30.3	6.7	32.9	11.4	30.8	10.9	32.0	11.0
(% EI)	(13)		(14)		(13)		(14)	
Polyunsaturated fat (g) (%EI)	5.8 (2)	2.6	5.4 (2)	3.4	6.3 (3)	3.3	5.7 (3)	3.3
Polyunsaturated/ Saturated ratio	0.2	0.1	0.2	0.1	0.2	0.2	0.2	0.1
Cholesterol (mg)	338.6	142.0	361.4	156.5	325.0	138.9	347.2	149.8
Sodium (mg)	1554.2	241.6	1854.3	753.5	1927.5	721.5	1860.4	722.5
Calcium (mg)	512.7	170.7	625.3	246.2	685.9	348.7	653.7	309.6
Iron (mg)	10.8	2.7	9.5	3.1	10.3	3.4	10.1	3.2
Vitamin A (ug Retinol)	400.2	120.9	932.9	375.2	590.2	363.0	779.2	288.3
Niacin (mg)	8.6	3.2	8.5	3.9	8.8	3.6	8.6	3.7
Fibre (g) Ψ ♠	8.7	2.6	8.5	3.8	6.8	2.9	7.9	3.5

Ψ p <0.05 low-income school compared with upper-income school

♠ p <0.05 upper-income school compared with middle-income school

Anova test

Comparisons between girls from the different schools showed that total energy (p= 0.036), carbohydrate intake (p= 0.000) and percentage of energy provided by carbohydrates (p= 0.002) and fat (p= 0.032) were significantly higher in girls from the middle-income school compared with the upper-income girls (Table 2.2). This is

partly because middle-income schoolgirls consumed significantly more sugar and sweets (p= 0.020) and fats and oils (p= 0.000) than upper-income girls. The average of all the schools together showed that cereal and cereal products provided the highest percentage of energy in relation to the total energy intake (29%), followed by meat and eggs (19%) and oils and fats (12%).

When comparing the data of all the schools together with the recommended values (Table 2.3), it can be appreciated that the diet of schoolgirls was deficient in calcium, polyunsaturated fat, iron, fibre and niacin. Iron intake was significantly higher (p= 0.000) in girls with the highest energy intake. Energy intake in upper-income and low-income girls was below the EAR.

The RNI for fibre was reached by only 6% of schoolgirls. The low consumption of fruits and vegetables and consequently of fibre, was more pronounced in upper class girls. In all groups fat intake was excessive (greater than 30% of total EI), as were saturated fat, cholesterol and sodium intake. 55% of the girls reported adding salt to the food on the table. This means that sodium intake exceeded the recommendation by an even greater amount. Vitamin A intake was above the RNI in the middle-income school and markedly below it in the low-income group. Vitamin C, folate, B1 and B2 were within recommended levels.

Briefly, in the whole group of girls, fat intake was high because of the high consumption of meat, eggs, oils and fats, while fibre intake was low as a result of the low consumption of vegetables and fruits. This dietary profile leads to an elevated cardiovascular risk. In addition, an important number of girls, especially from the upper and low-income group reported a low energy intake and consequently inadequate micronutrient intake. This feeding pattern is a characteristic of patients with AN (Van Binsbergen et al 1988; Hadigan et al 2000) and represents a susceptibility to eating disorders.

Table 2.3: Recommended daily intake for energy, macro and micronutrient

Energy intake (EI) (kcal) (MJ)	2110 (8.83)	*2
Carbohydrates (% EI)	55	*1
Protein (g)	45	*3
Fat (% EI)	<30	*1
Saturated fat (% EI)	<10	*1
Polyunsaturated fat (% EI)	6	*3
Polyunsaturated/saturated fat ratio	0.45	*1
Cholesterol (mg)	<300	*1
Sodium (mg)	1600 or 3 g salt	*3/*1
Calcium (mg)	800	*3
Iron (mg)	14.8	*3
Vitamin A (ug Retinol)	600	*3
Vitamin C (mg)	40	*3
Vitamin B1 (mg)	0.4	*3
Vitamin B2 (mg)	1.1	*3
Niacin (mg)	14	*3
Fibre (g)	15	*3
Folate (µg)	200	*3

*1 WHO (1990)

*2 The EAR of food energy for females between 15 and 18 years. EAR is an estimated average requirement of a group of people for energy or protein or a vitamin or mineral. Dietary Reference Values for UK (1991).

* 3 RNI: reference nutrient intake for protein or a vitamin or mineral for females between 15 and 18 years. RNI is an amount of nutrient that is enough for about 97% of people in a group. Dietary reference Values for UK (1991).

2.3.2.2. Meal intake

Table 2.4 Number of meals eaten by type of school

Number of meals	Low-income school			Middle-income school			Upper-income school			All schools together		
	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD
Out of home (weekly) Ψ ♠	*10*	4.2	3.6	*91*	4.2	2.8	54	10.9	4.7	*155*	6.4	4.8
With family (weekly) Ψ ♠ ∝	*10*	13.3	7.0	*91*	18.3	7.0	54	14.3	5.0	*155*	16.7	6.7
Main meals (daily) Ψ ♠ ∝	*46*	2.5	1.5	*123*	3.2	1.0	173	3.7	0.7	*342*	3.4	0.9
Snacks (daily)	*46*	2.5	1.5	*123*	2.1	1.2	173	2.0	1.2	*342*	2.1	1.3

Anova test

Ψ p <0.05 low-income school compared with upper-income school

∝ p <0.05 low-income school compared with middle-income school

♠ p <0.05 upper-income school compared with middle-income school

Interesting differences in the meal pattern were observed among the schools. Approximately 27% of all the girls omitted breakfast on the day of the study. The upper-income schoolgirls had significantly more meals out of home compared with middle (p= 0.000) and low-income girls (p= 0.000) (table 2.4). Middle-income girls had a significantly higher number of daily main meals (breakfast, lunch, tea and dinner) than the low-income girls (p= 0.000). Middle-income teenagers also ate significantly more meals with the family than low (p= 0.001) and upper-income girls (p= 0.048). Family meals significantly and positively correlated with energy intake (p= 0.000, r 0.303 Pearson correlation) and the number of total daily meals (p= 0.002, r 0.424). Therefore, sharing meals with the family may increase food consumption, protecting girls from dietary deficiencies. Besides, refusing to eat with the family is a key characteristic of patients with AN (Touyz and Beumont 1985), and is conducted to avoid eating and lose weight. The girls who were not eating socially might have been masking a dieting behaviour.

Coffee and alcohol intake and dieting did not differ between the girls who had at least one meal out of home and those who always ate the main meals at home

during the study week. Hence, the hypothesis that girls eating away from home would make more use of coffee and alcohol and would be more likely to be dieting, since they would be less controlled by their families, was not accepted.

2.3.3. Lifestyle behavioural patterns related to chronic diseases

Table 2.5: Lifestyle behavioural patterns associated with AN and cardiovascular disease by type of school

Behavioural pattern	Low-income school (%) N= 46	Middle-income school (%) N= 123	Upper-income school (%) N= 173	All schools together (%) N= 342
Having two or more cups of coffee daily	22	13	11	15
Drinking alcohol during the study week	39	32	28	33
Vegetarianism	2	4	2	3
Smoking tobacco	37*	19	19	25
Exercising less than three times a week	83	90	83	85
Dieting to lose weight during the study year	22	36	58*	39

*$p<0.05$ Chi^2

2.3.3.1. Coffee intake

On average 29% of the girls avoided coffee intake and this percentage was greater in the upper-income schoolgirls (39%). The majority of the girls consumed either less than a cup, or one cup of coffee daily. However, a considerable percentage of the low-income girls (about 22%) drank 2 or more cups of coffee daily (Table 2.5). Instant coffee was the most popular drink among girls and low-income girls mostly selected boiled coffee, which is the most damaging type.

2.3.3.2. Alcohol intake

Alcohol intake was more common in the low-income schoolgirls, where more than a third of teenagers consumed alcohol once or twice during the study week. Alcohol drinking was less popular among the upper socio-economic group, where most of the girls drank alcohol only once during the study week (Table 2.5). The mean alcohol intake in a total of 106 girls, who declared drinking alcohol during the study week, was 478 ml, SD 520. This amount equals more than ¾ of a pint of beer, however a great variability in the amount of alcohol drunk was observed.

2.3.3.3. Tobacco smoking

25% of the girls used tobacco (Table 2.5). The hypothesis that tobacco smoking in adolescent girls was associated with having a single parent was not proved. Powerful connections between the smoking habit and smoking by family and friends were noticed. The consumption of tobacco was positively associated with having a mother ($p= 0.018$, x^2 5.6 Pearson Chi^2 test), a brother ($p= 0.016$, x^2: 5.7), a sister ($p= 0.000$, x^2 17.2) or a close friend ($p= 0.000$, x^2 44.9) who smoked. Low-income girls had a greater percentage of brothers, sisters and close friends who consumed tobacco and 78% of these girls lived with one or more smokers. The majority of the all the schoolgirls (59%) had one or more persons smoking at home.

Girls who smoked tobacco had significantly higher BMI ($p= 0.005$, mean 22.1 kg/m^2 SD 3.1 T test) compared with those who did not (mean 20.9 kg/m^2, SD 2.6). Similarly, smokers had significantly fewer daily meals ($p= 0.000$ mean 3.1, SD 1.0 T-test), than those who did not smoke (mean 3.5, SD 0.9). Smoking was also negatively associated with dieting by skipping meals ($p= 0.004$, x^2 -8.1 Pearson Chi^2 test). This means that girls who smoked were eating less frequently than those who did not smoke. Positive associations were also obtained between tobacco and alcohol intake ($p= 0.000$, x^2 18.6 Pearson Chi^2 test). Girls who performed exercise were significantly less likely to smoke ($p= 0.001$ x^2 10.5 Pearson Chi^2 test).

2.3.3.4. Vegetarianism

Vegetarianism in the schools was almost non-existent, with only 3% of the girls being vegetarians (Table 2.6). However, red meat was avoided by an important number of upper-income girls and fish was avoided by nearly half the girls (Table 2.7). The avoidance of meat may mask dieting behaviour, leading to nutritional deficiencies. Moreover, vegetarianism is a usual choice of patients with AN (O'Connor et al 1987; Hadigan et al 2000; Matzkin et al 2001).

Table 2.6: Percentage of girls avoiding meat and meat products by type of school

Meat and meat products	Low-income school (%) N= 46	Middle-income School (%) N=123	Upper-income School (%) N= 173	All schools (%) N= 342
Red meat	10	8	17	12
Chicken	4	5	2	4
Fish and sea food	39	41	48	42
Milk and milk products	3	7	9	6

2.3.3.5. Exercise

Above all, two terms need to be clarified: exercise and physical activity. Exercise is defined as a planned, structure and repetitive body movement done to improve or maintain one or more components of physical fitness, while physical activity is understood as any body movement produced by skeletal muscles that results in energy expenditure (Gregory et al 2000). This study aimed to assess exercise levels.

Theoretically, as part of the school curricula, all the students take two classes of 45 minutes of exercise weekly. In the state schools these classes were not intense and girls usually played volleyball (a team game), while in the private school physical activity classes were optional and girls usually swam. The vast majority of girls

engaged in exercise only once or twice during the study week. However, nearly 20% of the teenagers were inactive (Table 2.5). People who perform little exercise are more vulnerable to suffer from the consequences of chronic diseases such as cardiovascular disease and osteoporosis. With regards to exercise duration, most of the girls exercised between ½ and one hour, although an important percentage of the upper-income schoolgirls (43%), exercised for more than an hour every time they exercised. Girls who exercised had a significantly greater BMI (p= 0.001 mean 21.4 kg/m², SD 2.9 T-test) than those girls who did not (mean 20.1 kg/m², SD 1.9).

2.3.3.6. Dieting

Dieting to lose weight was more prevalent in the upper-income schoolgirls and less usual in the low-income schoolgirls (Table 2.5). Notably, 10% of the upper-income girls had started a diet four or more times during the study year, suggesting a chronic dieting behaviour.

Dieting was positively associated with the intake of skimmed milk (p= 0.047, x^2 10.9 Pearson Chi² test) and with not adding sugar to coffee or tea (p= 0.001, x^2 10.2). These two dieting behaviours, probably chosen to lose weight, were again more frequent among girls from the upper socio-economic class. Girls who dieted had a significantly lower EI (p= 0.047, mean 2040, SD 513 T- test) than non-dieters (mean 2222 kcal, SD 593).

Table 2.7 shows the methods used to lose weight. In contrast to what was hypothesised, the most common method to lose weight was eating less food, followed by increasing physical activity and by eating less fat-rich food. However, skipping meals was a practice chosen by 28% of dieters. It is worth noting that upper-income girls chose nutritionally sound methods to lose weight, such as increasing physical activity and reducing fat-rich food. This may be because girls in the upper socio-economic class have better education on nutrition related topics. This presumption is based on the fact that adolescents from wealthier families in Buenos Aires have more knowledge about nutrition than teenagers from lower socio-economic groups (McArthur et al 2001). In total 10% of the girls used some of the unhealthy methods

to lose weight such as vomiting, laxatives, diuretics and slimming pills. Vomiting was more prevalent in the middle-income girls and laxative use was frequent in the upper-income and low-income teenagers.

Table 2.7: Methods to lose weight by type of school

Methods to lose weight	Low-income School (%) N=46	Middle-income School (%) N= 123	Upper-income School (%) N= 173	All schools (%) N =342
Eating less food	46	55	63	55
Strict dieting	9	15	9	11
Fasting	20	19	12	17
Food supplement use	6	2	1	3
Increasing physical activity	37	37	70*	48
Skipping meals	24	37	24	28
Reducing fat rich food	22	37	70*	43
Vomiting	4	6	3	4
Diuretic use	2	2	1	2
Laxative use	4	1	5	3
Slimming pill use	0	2	2	1

*$p<0.05$ Chi^2

Body satisfaction

Only a minority of girls were happy with their current body weight. The majority of the upper-income girls wanted to lose weight and a very small percentage wanted to put on weight (Table 2.8). Body dissatisfaction (rating as the options happy or unhappy with current weight) was positively associated with dieting to lose weight (p=: 0.000, x^2 15.3 Pearson Chi^2 test) and with attempts to lose weight by: eating less food (p= 0.000, x^2 30.0), decreasing fat intake (p= 0.000, x^2 25.0), skipping meals (p= 0.000, x^2 11.2) and increasing physical activity (p= 0.000, x^2 32.1). As was expected, body dissatisfaction was also linked to percentage of body fat and BMI (p: 0.000 and p: 0.000, respectively, T-test). The girls who were unhappy with their weight had a

significantly higher BMI (mean: 21.5 kg/m², SD: 2.7) than those girls happy with their weight (mean: 18.3 kg/m², SD: 1.3).

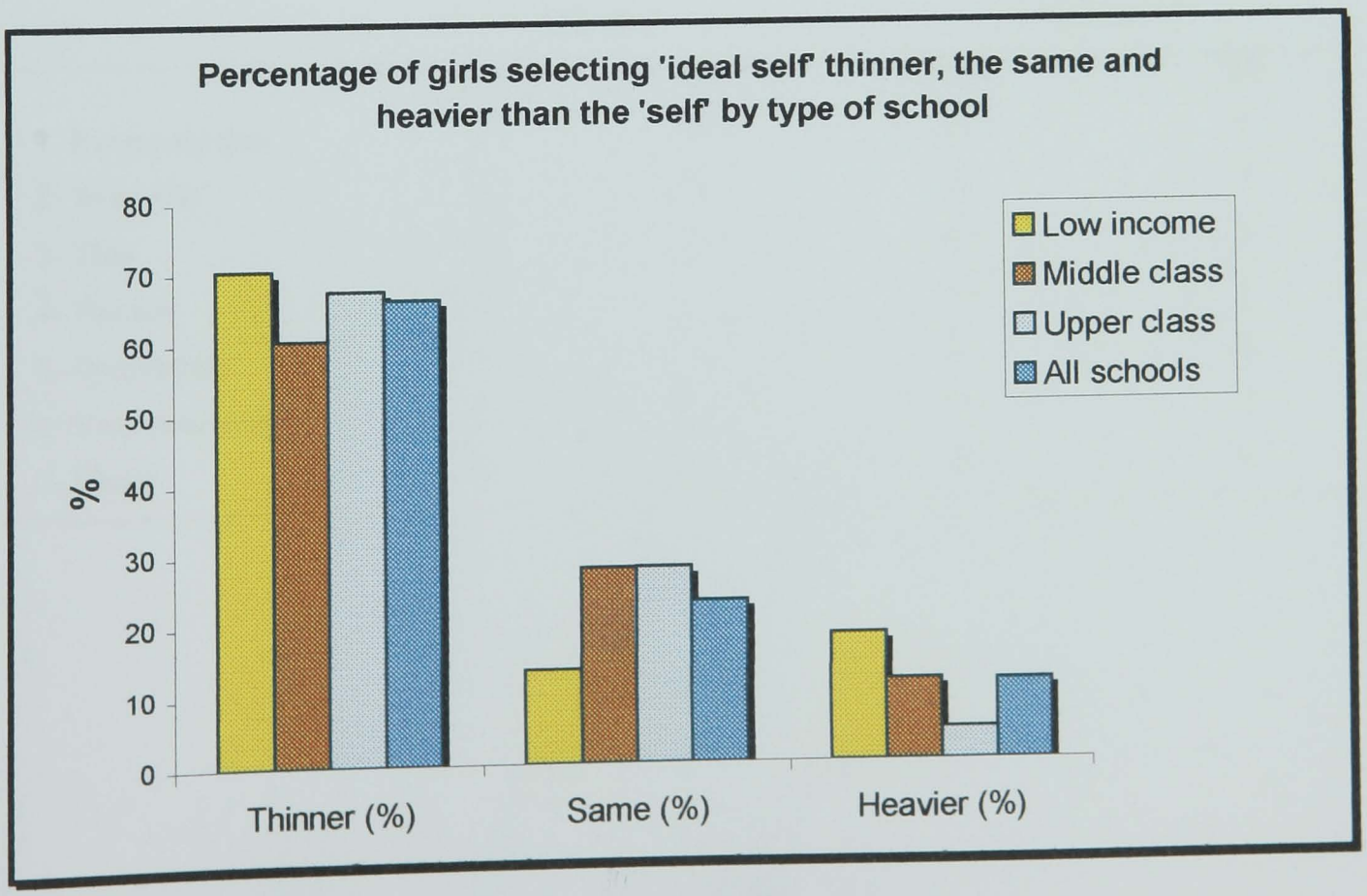
Table 2.8: Body weight satisfaction by type of school

Body satisfaction	Low-income (%) N= 46	Middle-income (%) N= 123	Upper-income (%) N= 173	All schools (%) N= 342
Put on weight	15	16	3	11
Lose weight	59	54	72*	62
Happy with current weight	26	30	25	27

*p<0.05 Chi² Test

No associations were found between weight dissatisfaction and coffee and alcohol intake, vegetarianism, low meal frequency, tobacco and unhealthy behavioural patterns to lose weight (vomiting, laxatives, diuretics, etc.). Therefore, the hypothesis that these behavioural patterns were linked to body dissatisfaction cannot be proved. However, the lack of association may be related to the small number of these weight-losing behavioural patterns among the scholchildren.

Graph N 2.2



SELF: Which picture looks most like you?

IDEAL SELF: Which picture shows the way you want to look?

More than half of the girls in all the schools wanted to look one figure smaller in the scale than the rated figure most similar to theirs. This indicates dissatisfaction with body size. Even more preoccupying is the finding that many girls, especially in the low-income school, wanted to look two or more figures smaller than the figures rated as those they most resembled. It is interesting to note that almost none wanted to look like Figures 5, 6 and 7 (Graph 2.2 and Figure 2.2).

When girls were asked to select the figure that most looked like them (SELF), most of the girls showed an agreement between their BMI and the figure chosen; that is, most of the thinner girls selected the thin figures and the heavier girls the heavy figures. In contrast, heavier girls selected thin figures and thinner girls selected heavy figures when asked to chose the figure they wanted to look like (IDEAL SELF) (Table 2.9).

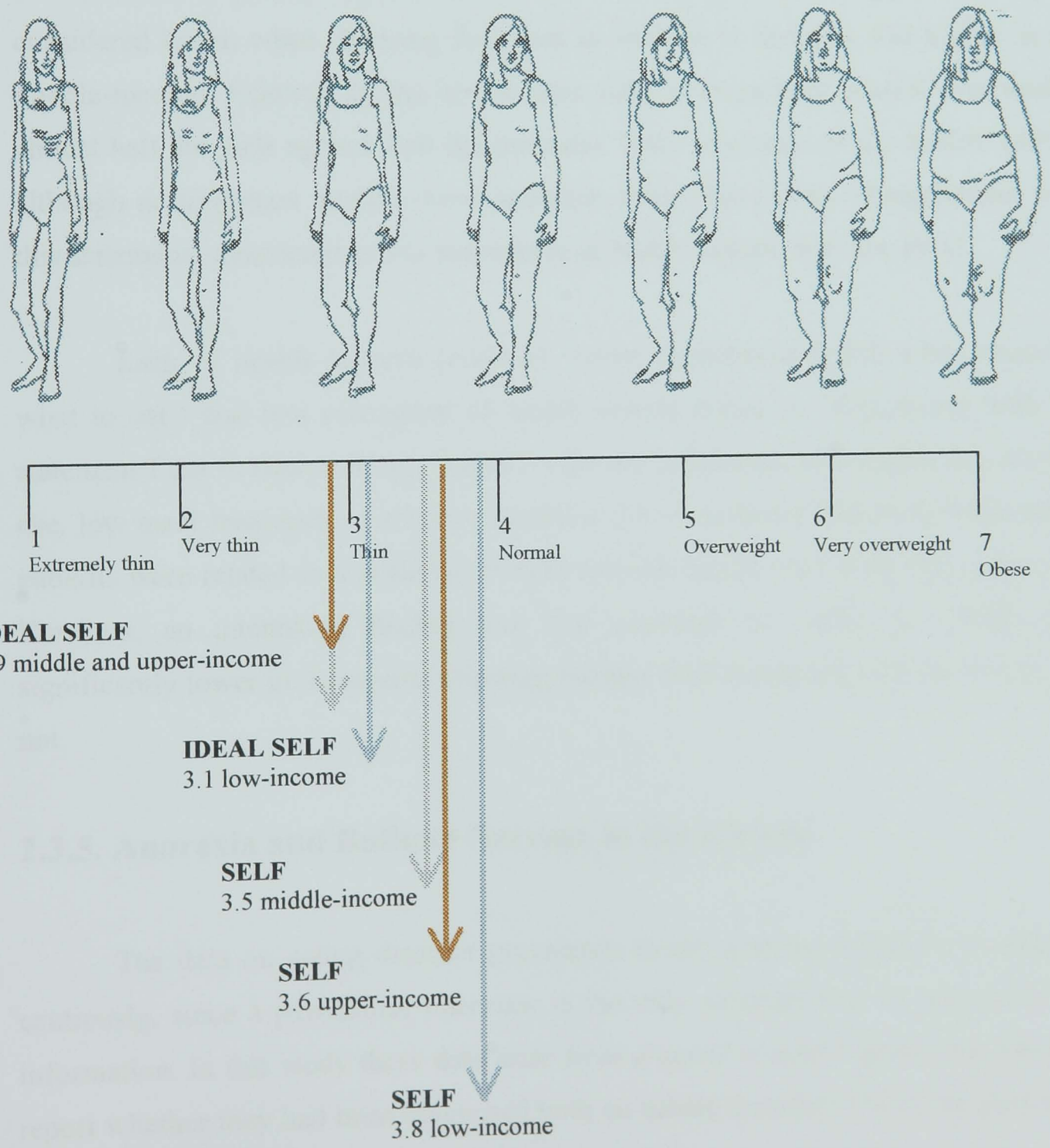
Table 2.9: BMI of the girls (kg/m²) by selected figure in the self and ideal self

Figures	Self Which picture looks most like you?		Ideal Self Which picture shows the way you want to look?	
	Mean	SD	Mean	SD
1- Extremely thin	17	0	21	1
2- Very thin	18	1	20	2
3- Thin	20	2	21	2
4- Normal	22	2	22	4
5- Overweight	24	3	31	0
6- Very overweight	27	3	21	3
7- Obese	34	0	0	0

Figure 2.2: Mean values for 'self' and 'ideal self' by type of school

SELF: Which picture looks most like you?

IDEAL SELF: Which picture shows the way you want to look?



2. 3.4. Attitudes and beliefs towards health

Two questions related to attitudes and beliefs about health (1.When choosing what to eat do you consider your health? and 2. Are you in control of your health?) were presented to the girls. It was assumed that those girls who worried about the quality of food eaten and those who had a sense of control over health would had a more positive attitude towards health and, therefore, would have been less likely to engage in risk behaviour leading to chronic diseases.

Almost half the girls (47%) only 'sometimes' considered their health when selecting what to eat. An important number of girls from the upper-income group (22%) stated that they did it 'very often'. The proportion of girls who never considered health when choosing food was in total 16 % and this was higher in the middle-income, followed by the low-income girls. In regards to control over health, almost half the girls agreed with the principle 'I am in charge of my health' (49%), although an important number were uncertain about this (35%). Disagreement with this statement, considered as low perception of health control, was low (6%).

Lack of health concern (rated as 'never considering health when choosing what to eat') and low perception of health control (rated as 'disagreeing with the statement I am in charge of my health') were not associated with coffee and alcohol use, low meal frequency or tobacco smoking. The hypothesis that these behavioural patterns were related to a negative attitude towards health was, therefore, unproved. However, an interesting finding was that saturated fat intake (p: 0.041) was significantly lower in those girls selecting healthy food compared with those who did not.

2.3.5. Anorexia and Bulimia Nervosa in the schools

The data on eating disorder prevalence in the schools should be considered cautiously, since a psychiatric interview is the only accurate way to determine this information. In this study these data arise from a question where girls were asked to report whether they had been diagnosed with an eating disorder. 1% of the girls (one

girl in each school) reported having been diagnosed with AN. The occurrence of BN in the schools was 2%. Five girls out of 342 (three from the upper-income school, one from the middle and one from the low-income school) reported had been diagnosed with BN.

2.4. Discussion

Different rates of participation were obtained for each of the tests; this means that not all the girls who completed the questionnaire had anthropometry taken and food diaries completed and explains the different sample sizes. The study may be bias because girls with disordered eating behaviour, underweight or overweight girls might have avoided participating and subsequently may be under-represented.

2.4.1.Anthropometric data

On average, the girls had a healthy body weight and a normal BMI, with girls from the low-income group being heavier. These data indicate a relative lack of obesity among these adolescents. With regard to the underweight girls, nearly a third had BMI values below the normal range and a considerable number had weights below the centile 25. This pattern was more prominent in the upper and middle socio-economic groups. Low body weight is a core characteristic of patients with AN and a risk factor for nutritional deficiencies and osteoporosis (Nelson 1993).

2.4.2. Food intake

2.4.2.1. Energy and macronutrient intake

Papers reporting energy and macronutrient intake within the age range of the adolescents in this study were reviewed. Despite the limitation in comparing studies such as different assessment methods of food intake and varied age groups, an attempt to summarise studies was made in Table 2.10:

Table 2.10: Studies reporting dietary intake in young females

Author and country	Sample size	Age group (years)	Assessment method	EI (kcal)	Protein (% EI)	Fat (% EI)	Carbohydrate (% EI)	Simple sugar (% EI)
Cook et al (1973) *England*	81	15	7-day weighed food diary	2060	11	-	-	-
Barker (1989) *Northern Ireland*	110	16-29	7-day weighed food diary	1820	13	40	47	-
Department of Health (1989) *England*	461	14-15	7-day weighed food diary	1890	12	38	51	-
Gregory et al (1990) *England*	189	16-24	7-day weighed food diary	1700	13	38	49	20
Crawley (1993) *England*	2754	16-17	4-day food diary	2105	11	42	47	21
Robinson et al (1999) *England*	47	14-16	Food frequency questionnaire/ Food checklist/7-day weighed food diary	2083	12	37	51	-
Gregory et al (2000) *England*	210	15-18	7-day weighed food diary	1622	14	35	50	15.8

From the analysis of the above studies the following results were gained: women had an average energy intake of 1897 kcal/d. Carbohydrate intake contributed 49% of total energy intake, protein 12%, fat 38% and simple carbohydrate 19%. Taking these studies as an example, it can be concluded that the diet of the schoolgirls contains:

- Low energy intake for the age and sex (the EAR is 2110 kcal)
- An excessive intake of total fat (the average percentage of energy from fat is above the recommended value of less than 30% of total EI).
- High percentage of simple sugars (recommended level is 10% total EI, Dietary Reference Value UK, 1991) and deficient complex carbohydrates (recommended level is 55% total EI, WHO 1990).

Nelson (1993) analysed seven studies reporting energy intake in adolescents and found that girls aged 16 years only achieved 80% of the energy reference value,

indicating that they were on a low-energy diet. The British National Survey of schoolchildren's diets (Gregory 2000) suggested that at least 5% of girls aged 14-15 years were dieting in order to lose weight. The same dietary pattern was observed in the girls from the present study where total energy intake was just adequate in the middle-income girls and deficient in the other two groups of schoolgirls. Energy supply from carbohydrates was low and the proportion of total daily energy supplied by fat was elevated. Dieting and low energy intake was common in upper-income schoolgirls. The current method of dieting was done through reducing carbohydrate intake since it was found to be significantly lower than in the other two sets of schoolgirls.

Dietary cholesterol and saturated fat were above recommended values (WHO, 1990). Epidemiological studies showed that those countries with higher saturated fat intake tent to have the highest rates of cardiovascular disease (Shaper 1988). The polyunsaturated to saturated fat ratio was reduced. Polyunsaturated fats are more susceptible to oxidation than saturated fats because they contain more than one double bond in the fatty acid chain. However, polyunsaturated fats reduce LDL levels preventing cardiovascular disease.

Crawley (1993) found that only 10% of females achieved the fibre recommended intake of more than 18 g. Nelson (1993) noticed low dietary intake of fibre in schoolgirls, partly due to insufficient vegetable consumption. Similarly, the fibre intake in the schoolgirls was below the recommendation, with only 6% of the girls meeting the RNI. Vegetable and fruit consumption was poor, contributing negligibly to the total energy intake. Fibre mainly acts on the intestine, lowering total cholesterol and LDL levels and its consumption is beneficial for the prevention of cardiovascular disease (Kromhout et al 1982).

To conclude this section, it can be stated that young girls show the same unhealthy food patterns, leading to long-term diseases, as those shown by adults. These main unhealthy dietary choices included high-saturated fat and low fibre intake.

2.4.2.2.Vitamin and mineral intake

Nelson (1993) explained that adolescents are falling short of the recommendation for dietary calcium, predisposing to bone malformation, fractures and osteoporosis in adulthood. In agreement with Nelson's study, the present study found low intake of calcium in schoolgirls. Calcium intake, through the consumption of milk and milk products, was greater in upper class girls and this may be due to a better nutrition education in this group.

This study confirms the observation of Pacin and co-workers (1999) who detected a low intake of dairy products and a consequent low calcium intake in adolescents from Buenos Aires. The low supplies of Calcium may contribute to the high incidence of bone fractures due to osteoporosis in Argentinean women (Mosquera et al 1998).

Deficient iron intake and anaemia are other recognised world problems, especially in women. Adolescents are at greater risk of iron deficiency because of the requirements for growth at this stage of life. Iron deficiency anaemia has been shown to affect the cognitive function in schoolgirls (Nelson et al 2001). Iron intake in this study was below the desirable levels. Higher, but not sufficient, iron intake due to increased meat and egg consumption was observed among the low-income girls. Because iron is mainly present in meat products, certain eating features adopted by adolescents such as restricting food intake, vegetarianism and red meat avoidance promote iron deficiency. Barber and co-authors (1985) noticed that the British young women who restricted their food intake were generally iron deficient. Similarly, the girls in this study who reported a lower energy intake had also lower iron intake. Therefore, adequate energy supply and meat and egg consumption are protective factors for iron deficiency.

2.4.2.3. Breakfast and daily meals

About one-third of the girls skipped breakfast on the day of the study. It has been demonstrated that subjects who skip breakfast have lower performance, poor memory and worse mood (Pollitt and Mathews 1998). Besides, the habit of skipping breakfast may lead to nutrient inadequacies such as calcium deficiency. Evidence exists that people who have a daily breakfast have more adequate intake of vitamins and fibre (Tietyen and Fleming 1995). Also when breakfast is skipped the consumption of out of home snacks, sweets and non-nutritious food probably arises.

Balding (1999) carried out a massive study on British schoolchildren and observed that 21% of the girls had nothing at all for breakfast the day of the study. He found associations between skipping breakfast and the desire to lose weight, although no association was achieved in this study. The omission of breakfast may be due to a lack of time or organisation to prepare it, or may be deliberately planned to reduce energy intake in those girls who want to lose weight. It could also be a cultural habit, as the Argentineans tend to have a little breakfast, a bigger lunch and a larger dinner.

Notable differences in the meal patterns were observed. Girls from the low-income school have a lower meal frequency than the other groups. A low meal frequency increases plasma total cholesterol and LDL levels. Higher cholesterol concentrations, a risk factor for cardiovascular disease, have been found in individuals consuming large meals each day compared with those consuming the same amount of food but in smaller meals (Jenkins et al 1989; Arnold et al 1993; Mcgralth and Gibney 1994.).

Energy intake was greater in those girls sharing more meals with the family. The state school timetable makes it possible to the girls to have lunch with their families as they attend school for half a day, either in the morning or in the afternoon. Girls from the most privileged socio-economic group ate more meals away from home. This may reflect the fact that they spent more time in the school and shared more meals with their peers. Middle-income teenagers shared more meals with their family, which may give the family members the opportunity for conversing and

relating to each other. Socialised behaviour in participating in formal 'sit down' meals that take place at regular intervals at home protects the girls from nutritional deficiencies (Branner et al 1994). Girls living with both parents have more adequate energy intake, indicating that a traditional family set up may be beneficial for adolescent development and health.

A distinctive finding is that patients with AN have a reduced frequency of daily meals and hot meals (Van Binsbergen et al 1988), which are usually eaten in a family dinner. In AN, there is also an avoidance of any social occasions where eating is involved (Touyz and Beumont 1985). Therefore, these dietary patterns shown in adolescents, as are also practised by subjects with AN, would represent a susceptibility to an eating disorder.

2.4.3. Lifestyle behavioural patterns related to chronic diseases

Not many adolescents experience significant levels of disease, on the contrary most of the illnesses in adolescents are behaviour related. So, health-risk behaviour including alcohol, tobacco and unhealthy weight-control methods should be targeted. Unhealthy behavioural patterns cluster and reinforce each other. Benthin and colleagues (1993) observed that teenagers who engage in risk activities reported greater knowledge of risk and less fear of risk. Adolescents who engage in one risk behaviour also tend to engage in another (Sutherland and Willner 1998; Benthin et al 1993). This study confirms this observation, because the girls who drank alcohol were also more likely to smoke tobacco and the girls who smoked tobacco were less physically active. Six dietary and behavioural risk patterns related to chronic diseases will be discussed below: 1. Coffee intake, 2. Alcohol intake, 3. Vegetarianism, 4. Tobacco, 5. Exercise and 6. Dieting and body dissatisfaction.

2.4.3.1. Coffee intake

The majority of the girls consumed coffee (71%) though upper-income teenagers consumed it with less frequency. Coffee intake was lower in the recent survey of British girls (Gregory et al 2000), with fewer than half the girls (48%) from 15 to 18 years old drinking coffee. In the present study, the low-income girls were more likely to suffer the harmful effects of coffee. A high number of this group of girls consumed two or more cups of coffee daily and boiled coffee was the most popular beverage. The harmful effect of excessive coffee intake (two or more cups a day) is that it has been linked to the development of fractures and osteoporosis (Hernandez Avila et al 1991).

Excessive coffee consumption may also contribute to the development of cardiovascular disease. LaCroix and colleagues (1986) carried out a 30-year follow-up study in a group of students and found that subjects who drank five or more cups of coffee per day had the highest incidence of cardiovascular disease. There are other components of coffee, rather than just caffeine, which may contribute to cardiovascular disease risk. A Scandinavian study suggested that, during the preparation of boiling coffee, hypercholesterolemic fractions are generated (Zock et al 1990). The lipid-rich supernatant of boiled coffee obtained after centrifugation raised serum LDL and triglyceride levels.

Many patients with AN consume a large amount of drinks containing caffeine (coffee, tea and diet coca cola), which provide few calories and can suppress the appetite (Sours 1983; Forman et al 1997). Therefore, adolescents who consume excessive amounts of coffee to lose weight may be more vulnerable to a number of diseases, including eating disorders, cardiovascular disease and osteoporosis.

2.4.3.2 Alcohol intake

A third of the girls consumed alcohol and this figure was higher in low-income groups. The alcohol drinking habit is generally initiated by imitation of peer behaviour and/or by parental attitudes and behaviour towards alcohol. In Britain,

Balding (1999) found that half the schoolgirls studied consumed alcohol during the week of the study. In this study the consumption was lower, which may be explained by cultural differences, since alcohol drinking in Argentinean girls is less socially expected and, therefore, less subject to peer pressure. Although the consumption is not as high as Britain, alcohol use among schoolgirls is still a problem.

The finding that approximately 33% of girls consumed alcohol agrees with the Argentinean study of Moss and colleagues (1998). Alcohol drinking usually begins during young adulthood, but the consequences of excessive drinking do not necessarily become apparent until later in life. The intake of alcohol in Argentinean females may be a contributing factor to the high incidence of both cardiovascular diseases and osteoporosis.

Binge alcohol drinking, which is a common practice in adolescents (Taboada Areso 2000), is linked to cardiovascular disease because it increases blood pressure and blood lipid concentrations (Moreira et al 1998; Nanchahal et al 2000). Another effect of prolonged alcohol consumption is the loss of calcium from the bone, which may lead to osteoporosis (Moniz 1994).

2.4.3.3.Vegetarianism

In the present study only a small number of girls were vegetarian (3%), with a considerable number of the girls from the upper socio-economic level avoiding red meat (17%). A wealthier situation allows these girls different choices of food items. Notably, fish consumption was avoided by nearly half of the girls and this may be understood by cultural reasons, since fish is an expensive and unusual food in Argentina.

Vegetarianism is becoming popular among schoolgirls worldwide. Worsley and Skrzypiec (1998) studied the vegetarian tendencies of two thousand 16-year old students in South Australia and found a great prevalence of vegetarianism (ranging from 8 to 32%), especially in girls. The estimate of vegetarianism for the UK

population aged 16 years and over is 4%, rising to 10% if semi-vegetarianism is included (Beardsworth and Keil 1993).

Martins and colleagues (1999) suggested that the adoption of a vegetarian dietary style is an attempt to mask their dieting behaviour from others. Neumark-Sztainer and colleagues (1997) reported than 0.6% (N=107) of the children in the USA (Minnesota Adolescent Health Sudy) were vegetarians and 81% of these were females. Young vegetarian girls were more likely than non-vegetarians to engage in disordered eating behaviours such as vomiting, use of laxatives, diuretics and binge eating. Vegetarianism, in its various forms, has been reported in AN by several researchers (O' Connor et al 1987; Hadigan et al 2000; Matzkin et al 2001). The link between vegetarianism and eating disorders is therefore strong, as females may choose not to eat meat for weight reasons.

Beef consumption in Argentina has been decreasing for the last few years (Meehan and Katzman 2001). Beef consumption per/capita was 83 kg in 1986 and decreased to 58 kg in 1998 (Avery 1998). This is an unexpected fact, since Argentina is known to be a 'beef-eating' nation. The decrease in meat intake in Argentina may be partly a result of the avoidance of meat by an increasing number of women who diet in order to lose weight. The dangerous effect of unplanned vegetarian diets is that in the short-term it may predispose to energy, protein, vitamin B12 and iron deficiencies (Nathan et al 1996; Dwyer et al 1982) and in the long-term to related chronic diseases (Hung et al 2002).

2.4.3.4. Tobacco smoking

Adult smoking habits are formed during adolescence. Three-quarters of the adult smokers were smoking at the age of 18 (Marsh and Matheson 1983). Bolling (1993) reported that 10% of school students in England aged 11 to 15 years were regular smokers (defined as usually smoking one or more cigarettes a week). Also in England, Sheffield (1984) observed that the proportion of children smoking rises with age and by the age of 15 or 16 years one in four girls and one in five boys were smoking.

The present study confirmed that tobacco smoking, dieting, BMI, low energy intake, low meal frequency and skipping meals were related. It has been suggested that women regard smoking as a way of keeping slim (Graham 1993; Crisp et al 1999). This is illustrated by the high incidence of smoking among patients with eating disorders (Haug et al 2001; Crisp, 1999). Smoking becomes a way of avoiding eating, an appetite suppressant, an alternative comfort-oral activity and part of an aim to lose weight. It is well documented that smokers have a lower mean weight than non-smokers. Nicotine has been found to cause a marked increase in metabolic rate, both at rest and during exercise (Hofstetter et al 1986; Perkins et al 1989).

The harmful side of tobacco is that it has been widely associated with the incidence of sudden death, stroke and cardiovascular disease. Slone and colleagues (1978) noted that women who reported smoking more than 35 cigarettes per day had a 20-fold higher rate of myocardial infarction than those women who had never smoked. Tobacco promotes the thickening of the coronary arteries and atheroma. Smokers tend to have unfavourable lipid patterns and higher fibrinogen levels (Wilhelmsen et al 1984).

Goddard (1990) observed that there were pre-existing characteristics in schoolchildren who subsequently started to smoke compared with those who did not. These risk characteristics were: being a girl, having brothers or sisters who smoke, living with a lone parent, having less negative views about smoking, not intending to stay on to full-time education after the age of 16 years, thinking that they might be a smoker in the future. Some of the mentioned hypotheses have been tested in this study. One of the remarkable findings was that 25% of the girls smoked tobacco and the majority of the smokers consumed up to 10 cigarettes daily. Mostly girls who consumed tobacco had a close relative or friend who also smoked. Familial transmission appears to be influential in the girls' smoking practice and it may become a consequence inherited through generations. This study has demonstrated that the socio-economic background influences tobacco smoking. Girls belonging to the most deprived sectors were more inclined to smoke and to have family and friends who also smoke. As Balding (1999) pointed out, girls in smoking homes may tend to experience approval rather than toleration and grow up thinking that it is normal

behaviour. A worrying finding is that non-smoking households were slightly in the minority.

The Argentinean government carried out a study among young people (12 to 18 years old) to investigate the prevalence of smoking, led by the worrying fact that tobacco was responsible for 45,000 deaths per year (SEDRONAR 2001). The main finding was that 30% of adolescents from the country's most important cities were smokers. Miguez (2001) observed identical results in a massive sample of school-students from Buenos Aires and noted that 43% students reported that smoking helped them to lose weight. Consistent with these previous reports, the present study shows that an almost similar percentage of girls (25%) used tobacco and that there was an association between tobacco and dieting. It seems that some Argentinean girls are smoking tobacco to avoid eating and this may be one of the reasons explaining the increased incidence of cardiovascular diseases among young Argentinean females (Hauger-Kevene and Balossi 1987).

2.4.3.5. Exercise

Childhood physical activity is thought to promote adult physical activity (Armstrong et al 1990; Sallis 1992). In other words, girls who were active during infancy would be more likely to exercise during adolescence and adulthood. Effective exercise during early years and adolescence will guarantee a less sedentary lifestyle later in life and prevent many chronic diseases.

A commonly prescribed exercise practice is for at least 20 minutes three times per week. (American College of Sport Medicine 1985). More recently, the American College of Sports Medicine (1995) recommended 30 minutes or more exercise every day. Gregory and colleagues (2000) found that only 40% of the British schoolgirls were meeting the recommendation of the British Health Education Authority of one hour of moderate daily physical activity. The present study confirms this observation. Most of the girls exercised only once or twice a week, though upper-income girls exercised for a longer duration.

Epidemiological studies have shown that people with higher levels of physical activity experienced a lower risk of cardiovascular disease (Klapan et al 1985). The mechanisms by which exercise might protect against cardiovascular disease involve effects on coagulation and thrombosis, on lipid metabolism and on blood pressure (Nelson et al 1986). In addition, regular exercise encourages a healthy bone formation in adolescence (Turner et al 1992), protecting against future osteoporosis. Exercise may also be effective in the maintenance of desirable body weight, inducing energy expenditure and suppressing the appetite (King et al 1994).

Increasing physical activity was one of the favourite ways that girls chose to lose weight. Upper and middle-income girls were lighter than lower-income girls. Upper-income girls had almost identical energy intake to low-income girls, while middle class girls energy intake was higher. Exercise frequency and intensity may explain these differences in energy intake and body weight.

Discrepancy in type, frequency and duration of exercise by social socio-economic group may be due to many reasons. First, upper-income teenagers have greater access to health information, exercise facilities and diversity of food, all of which may influence the physical activity pattern. Secondarily, girls from the middle and the low-income group may not engage in vigorous exercise because of the lack of motivation from home, poor exercise facilities, working duties and lack of time and money to spend on exercise. The schools do not play a significant role in promoting exercise among girls. The frequency and intensity of exercise during physical education classes do not usually meet the recommendations for a healthy exercise pattern (Simons-Morton et al 1994). It is unlikely that girls, especially those from deprived sectors, would engage in frequent and effective exercise outside school hours.

Although data on exercise among Argentinean adolescents are scarce, there are some interesting studies. Martinez and colleagues (2001), for example, observed that secondary school females from northeast Argentina exercised less than 3 hours weekly. Bazan (2000) added more information on this topic, carrying out a physical activity assessment in schoolchildren (6 to 14 years) in different cities of Argentina.

84% of the girls were rated as being inactive, because they performed no exercise during their spare time, and they were taking only 1 and ½ hours per week of physical activity at school. The present study supports the findings of low exercise frequency in schoolgirls. Lack of exercise is a prevalent trend in Argentinean women and it is another factor to account for the high incidence of cardiovascular diseases and other chronic diseases.

2.4.3.6. Dieting and body satisfaction

Teenage girls are one of the age groups most preoccupied with their bodies and appearance. One way of dealing with social acceptance and self-esteem is by dieting to lose weight. Many girls who are a normal weight may also engage in harmful weight-reducing behaviour. The possible problems of dieting in adolescents are: 1- that dietary restraint may cause subsequent overeating and purgative behaviours, 2- that dieting has often been found to precede the onset of an eating disorder, 3- that it may delay the normal development in puberty, 4- that it predisposes girls to nutrient deficiencies with the consequent risk for osteoporosis and other chronic diseases in adulthood.

In Britain, Patton and co-workers (1990) commented that 31% of 15-year old London schoolgirls (N= 739) were dieting and that the relative risk for dieters developing an eating disorder was 8 times that of non-dieters. 1/5 of the dieters were classified as having an eating disorder when assessed by an interview 12 months later. 3% of the sample had an eating disorder. The prevalence of eating disorders in the school population was assessed in many other studies. Crisp and co-workers (1976) found one case of AN for every 200 schoolgirls. The disorder was more frequent in the upper social class and in girls averaging 16 years old. Johnson-Sabine and colleagues (1988) calculated the prevalence of eating disorders to be 1% and 2% for partial eating disorder syndrome in London schoolgirls. In the USA, Whitaker and colleagues (1990) estimated one case of AN for every 500 girls. In the present study the prevalence for AN was 1% and for BN 1.5% and the number of girls who were dieting (39%) was similar to the figures provided by Western studies.

Behavioural patterns to lose weight are detrimental for the young and may be precursors to eating disorders. In the American study of Neumark-Sztainer and colleagues (1999) the most common methods of dieting were increasing physical activity and disordered eating such as vomiting, diuretics, laxatives was reported by 7% girls. In the present study, the most used methods of dieting were eating less food followed by increasing physical activity and 10% of girls were using unhealthy methods for losing weight.

The present study found that the desire to lose weight and the frequency of dieting was higher in the upper SE group. Studies from developed societies often report that body concern is greater in higher social classes. Szmulkler (1985) pointed out that eating disorders are more frequent in girls from private schools than from state schools. Dornbusch and co-workers (1984) in a National USA sample of teenage females (n: 2,177) found a stronger desire to be thinner in the high SE income girls than in the middle and low income girls, even after adjusting for levels of fatness. Story and colleagues (1995) reported that Minnesota females aged 12 to 20 years (n: 16,852) of low SE status were less likely to be dieting, to view themselves as overweight, to vomiting or using diuretics than middle SE girls. Wardle and Marsland (1990) in London showed that females students (n: 439) from high SE schools were slimmer, more concerned about weight and dieting than those from low SE schools. Studies from less developed societies also show less obsession for slimness in lower social classes. Nasser (1986), for example, found that Arab students from London scored higher in the EAT that their counterparts in Cairo. Furnham and Alibhai (1983) also documented that African women rated larger body figures as more attractive than British women.

Although a prominent proportion of the low-income girls desired a thinner figure, the hope of losing weight and the frequency of dieting was notably lower than in the upper socio-economic group. A stronger family and peers weight-related pressure in the upper-income girls probably explains this. Another datum of interest in the study is that the girls from higher socio-economic level were using low-fat milk instead of whole fat milk and avoiding the addition of sugar to infusions. These two

food choices were related to dieting and as Barr (1995) commented they may reveal a dieting-orientated behaviour.

The greater the body dissatisfaction and exposure to dieting, the greater the risk of having nutrient deficiencies. The present study showed that only 27% girls were happy with their weight. Dieting and body dissatisfaction were associated with total body fat, suggesting that those girls with greater fat content were unhappy with their weight and more likely to be dieting and exercising. Heavier girls were sensitive to this issue and experienced a desire to change. Dieting in turn was associated with low energy intake, indicating that dieters were succeeding in their goal to reduce their food intake. Similarly, Neumark-Sztainer and colleagues (1999) in the USA and Zukerfeld and colleagues (1998) in Argentina observed that girls with the highest BMI were at increased risk of disordered eating behaviours. Therefore, heavier girls should be highlighted as being at a greater risk of dieting and eating disorders. This is an interesting observation because dieting is socially expected in the heaviest girls and generally only the thinner girls are seen at risk of eating pathologies.

This study demonstrated that girls who menstruated earlier were more prone to dieting and to be heavier. This finding supports the study of Blyth and co-workers (1985) which showed that girls who matured early were more dissatisfied with their weight and figure than those who matured late. Early maturity serves to increase dissatisfaction via the weight gain linked to puberty. Body dissatisfaction and dieting could therefore relate to the levels of child development. Girls who menstruate early may be less psychologically mature to fight against the problems of adolescence such as low body esteem, identity seeking, parental independence, etc. and more likely to dieting.

Low body esteem is an instigator of dieting to lose weight, and emaciated girls with AN expressed this severely (Brunch 1962). When patients with AN are asked -do you think you are fat or thin? -, They will report that they consider themselves fat even when underweight; they overestimate their body size. The concept of body esteem is formed by the individual perception of how members of our cultural group judge our body.

Dissatisfaction with the appearance may therefore be related to family ideals, the influence of peers and the cultural ideal of thinness. Maternal concerns with weight and shape serve as important modelling cues for a young adolescent girl's weight management (Collins 1996). Peer pressure and physical appearance related teasing are also considered relevant factors in the aetiology of body dissatisfaction and eating disorders (Striegel-Moore et al 2002). Thompson and Sherman (1993), working with sports subjects, commented about contagion effect in eating disorders and that pathologic weight loss methods can be learned from one athlete to another. In relation to cultural values, adolescent girls easily accept and internalise beliefs, stereotypes and prejudices against obesity (Hill et al 1992). Teenagers do not usually identify with obese people and a slim image may therefore be associated with greater success in life. This was demonstrated by the fact that almost none of the girls in this study wanted to look like the heaviest three figures of the body rating scale.

Although eating disorders are a serious problem in Argentina there is little literature available on this matter. Garcia and co-workers (1997), for example, reported the alarming finding that about 84% of the female students in central Argentina were preoccupied with their body and wanted to lose weight even when they were not overweight. Zuckerfeld and colleagues (1998) provided more data on the subject, observing an important frequency of dieting (30%) among Buenos Aires students. The present study has shown an even higher prevalence of dieting (40%) and discontent with their body (74%) among schoolgirls. Many Argentinean females have, therefore, low body esteem and this induces them to diet, with a resultant risk for nutritional deficiencies, eating disorders and other long-term health problems.

2.4.4. Attitudes and beliefs about health

Girls who cared about choosing healthy food had low saturated fat intake, demonstrating that the attitude towards eating healthy food affects the behaviour. Publicity and the new trend of eating non-genetically modified food and preferring organic products may also bear influence on girls' choice of food (Balding 1999), especially in the upper socio-economic group.

Nearly half the girls agreed with the statement- I am in charge of my health-. This suggests a positive belief towards health, as girls may think that risk behaviour can be changed in order to achieve healthier lifestyles. This is a good starting point to implement behavioural modification.

2.5. Limitations and recommendations

A number of limitations of this study should be mentioned:

1. The level of education may bear an influence on the information obtained by the food diaries and questionnaires and therefore the data should be considered with care.
2. Sample size is small
3. The food diary requires a high-level of motivation and skill and this may be a reason for incorrect recording of food intake.
4. The schools were not selected scientifically at random, however, the Sub-secretary of Education undertook the allocation of the schools based on the request of investigating schoolgirls from different socio-economic groups.
5. Many variables, for example exercise levels and diagnose of eating disorders were self-reported and should be interpreted with limitations
6. The prevalence for AN was around 1% and for BN 2%, although weight loss behaviour was reported by 10% of the girls. This discrepancy may be due to under-reporting eating disorder problems or the fact that many girls, and probably those with eating problems, failed to complete all the tests of the study or refused to take part. Therefore, the prevalence might have been higher. A well designed questionnaire, such as the EDI or the EAT, to search for eating disorders symptoms, followed by an interview to identify cases, would have been a more accurate method of assessing prevalence of eating disorders in the schools.
7. Since the study was voluntary, different rates of collaboration were obtained for each of the tests; this means that not all the girls who completed the questionnaire had anthropometry taken and food diaries completed. The consequent different sample size in the results may be a bias in the study. For example, those girls with

disordered eating behaviour or who were overweight might have avoided participating in parts of the study, such as anthropometry, because of embarrassment, or might not have answered honestly the questions for fear of being discovered. The food diary was completed by a small number of the low-income girls and, therefore, the sample size might not have been representative enough to make comparisons with the other two groups. The rate of dropouts in the study might have been prevented by taking random samples of the girls in each school and performing the tests in a smaller and representative sub-group.

8. This research was conducted in the city of Buenos Aires and, therefore, generalisations about the representation of the Argentinean population should be considered with care.

Further research on the following areas is essential to improve the knowledge of the behavioural problems in adolescents: 1. Incidence of eating disorders in Argentinean schools, 2. Risk perception of unhealthy behavioural patterns, 3. Factors contributing to body dissatisfaction such as peer pressure, bullying, parental beliefs, etc, on different socio-economic levels, 4. Accurate methods to assess physical activity, 5. Evaluation of prevention programmes to combat risk factors for chronic diseases.

2.6. Prevention

The type of prevention programmes to be implemented in the schools is 'primary prevention', which refers to programmes that are designed to prevent the occurrence of an unwanted behaviour before it begins. An understanding of the risk factors, or unwanted behaviour associated with eating disorders, is essential in the planning of preventive programmes. Research into the risk factors for eating disorders is still developing and a brief account of the risk factors was shown in Chapter 1.

There are methodological problems when evaluating the effectiveness of intervention programmes for eating disorders, due to different methods used, different target populations, risk factors targeted, measurement of outcomes, etc (Pratt and

Woolfenden, 2004). Some of the interventions to reduce eating disorders have produced successful results. Stice and colleagues (2002), for example, reported a decrease in thin ideal internalisation and bulimic symptoms after two prevention programmes (a healthy eating programme and a body image intervention) to high-risk females. However, there were no changes in body dissatisfaction and dieting following the interventions. Similarly, Killen and colleagues' (1993) prevention programme in schoolchildren resulted in an increase in the knowledge about eating disorders, but no effect on body dissatisfaction or weight control behavioural patterns.

Multi-session interventions have shown to be have greater impact than single-session interventions (Stice and Shaw, 2004). Also, eating disorder prevention programmes that target high risk population, for example adolescent girls, are more effective in reducing risk factors for eating pathology than universal programmes, which include all possible subjects (Stice et al 2002). High-risk subjects have probably more motivation to engage in the programme and experience the benefits. If this principle is applied to the results of this study, socio-economic level can be identified as a risk factor for much behaviour to be targeted. For example, smoking, alcohol, excessive coffee intake and low exercise duration were more prevalent in the low-income school, while desire to lose weight and dieting were more frequent in the most advantaged socio-economic group.

Prevention methods regarding eating disorders have been criticised, with the suggestion that they could be more harmful than helpful. Asking children about unhealthy dietary behavioural patterns might be perceived as an incitement. However, Pratt and Woolfenden (2004), in a systematic review of eating disorder prevention programmes, found a lack of evidence for harm being caused as a result of any intervention.

Most of the preventive programmes for eating disorders have been focussed on:

- Psycho education; targeting knowledge about eating disorders.
- Modifying eating attitudes and behaviour. Some of the educational topics include coping with the demands of growing up, unhealthy weight regulation practices,

promotion of weight regulation via nutrition and healthy exercise. Intervention programmes that involve increased physical activity, dietary education and reduction in sedentary behaviour are also used in the prevention of obesity (Campbell et al 2002).

- Encouraging critical analysis of the media and societal attitudes about body weight. Discussing the sociocultural and peer pressure to be thin, the social rejection of overweight people, and the intolerance of diversity.
- Promoting self-esteem while discussing topics such as dealing with stress, building a positive sense of self, positive self-evaluation, relationship and communication skills.

With regard to tobacco smoking, cigarette advertising seems to be directed at this age group (Pierce and Gilpin 1994). Anti-smoking campaigns are needed among adolescents, as well as treatment to encourage giving up smoking. Smoking is a damaging habit, which usually starts in adolescence, and is often maintained for life. This is an optimal time to teach about the risks of tobacco consumption. A successful smoking prevention programme in American schools encouraged student leaders to lead small groups of younger students in discussions that emphasised statements resisting the social pressure to smoke (McAlister et al 1979). Another point to take into account, when planning anti-smoking campaigns, is that adolescents are usually more affected by the immediate short-term consequences of smoking (limited endurance in sports, bad breath) than in the long-term consequences of smoking.

2.7. Conclusion

The purpose of this chapter is to provide an overview of the lifestyle habits, eating patterns and body conceptions that characterise young girls from different socio-economic backgrounds. Behavioural patterns are established at early ages and may be contributory factors towards chronic diseases later in life. The high incidence of cardiovascular disease and osteoporosis in Argentinean women may be a consequence of unhealthy life-styles from childhood and adolescence.

The schoolgirls were eating excessive amounts of saturated fat and cholesterol and low amounts of carbohydrate, iron, calcium and fibre. The appropriate nutrition of young women is important for bone and heart health, since they are still growing and their nutritional demands are high.

The expectation was to find more dietary inadequacies in the lower-income groups. Conversely, the upper-income schoolgirls showed a lower energy and fibre intake. Findings of this study add to the evidence that schoolgirls from all socio-economic levels are preoccupied with their body weight and shape. However, a high percentage of the upper socio-economic class girls were underweight and more concerned about body weight and dieting. This may be due to higher social pressure to achieve a slim body shape. Decreasing food intake and exercising were by far the most frequent methods adopted for losing weight.

The patterns of behaviours associated with cardiovascular disease such as, lack of exercise, smoking and alcohol were more prevalent in the low-income group. Smoking was probably used as a means to lose weight and was associated with energy deficiency and low meal frequency. There was a consistent tendency for unhealthy behavioural patterns to be adopted by low-income girls. So, any strategy to change these circumstances by reducing deprivation is more probable to decrease the risk factors. For example, the low-income girls were more likely to smoke because they lived in a smoker environment and they were probably less likely to exercise intensively because of a lack of opportunity, and more likely to drink boiled coffee because it was cheaper and readily available.

The schoolgirls from Buenos Aires were totally imbued with Western values of body shape and size. Adolescence is a risk period of great physical and emotional changes. Many body-dissatisfied teenagers may restrict their food intake or engage in unhealthy behaviour as a means of losing weight and feeling better. The prevention of chronic diseases should start early in life because risk factors for eating disorders and unhealthy behavioural patterns are prevalent in adolescents. A political plan of feasible strategies to prevent chronic diseases is crucial, and everyone involved; the

adolescents, the parents, the schools and the members of the government have an active role in this process.

Chapter 3 - Clinical notes review (Study 2)

Chapter 3 - Clinical notes review

3.1. Introduction

High concentrations of blood cholesterol have been documented in patients with AN (Chapter 1) and recognised as a cardiovascular risk factor, but the relationship between AN and cardiovascular disease is unclear. Data on cholesterol concentrations among patients with AN are inconsistent. Melher and colleagues (1988), for example, reported normal concentrations of cholesterol compared with reference values.

Higher total cholesterol values may be related to the age of the patients, the BMI and a feeding pattern characterised by overeating followed by vomiting (Blendis and Crisp 1968; Case et al 1999). The use of tobacco to control weight has been reported in patients with AN, particularly in the non-restricting type of patients (DSMI IV 1994), who do not regularly engage in binge-eating or purging behaviour (for example: self induced vomiting or misuse of laxatives, diuretics or oedemas) (Wiederman and Pryor 1996; Haug et al 2001). Tobacco smoking increases atherogenic blood lipids and is one of the main modifiable risk factors for cardiovascular disease (Task Force Report 1998). The amount of tobacco smoking determines the risk of myocardial infarction in young women (La Vecchia et al 1987). The hypercholesterolemia in patients with AN may, therefore, be explained partly by the use of tobacco.

Along with excessive exercise, constant hyperactivity is to be expected in AN, as it is one of the primary characteristics of the disorder, and these may be responsible for the increase in HDL values (Kaplan et al 1985).

This chapter aims 1. to compare blood cholesterol concentrations from a large sample of patients with AN with control subjects from a healthy population, and 2. to explore the relationship between cholesterol values in AN and BMI, the age of patients, vomiting, exercise and tobacco use. The study described in this chapter

reported blood level values in adolescent and adult Argentinean patients with AN and controls. It includes a larger sample size than previous cholesterol studies in AN and it is one of the few clinical assessments on Argentinean patients and on healthy young females from Buenos Aires.

Hypotheses

Primary Hypothesis

- Patients with AN have higher concentrations of blood total cholesterol at diagnosis than a sample of normal weight subjects matched for sex and age.

Secondary Hypothesis

- Blood total cholesterol concentrations in patients with AN are positively correlated with the age of the patients at diagnosis, occurrence of vomiting and use of tobacco.

- Blood total cholesterol concentrations in patients with AN are negatively correlated with BMI.

- HDL cholesterol concentrations in patients with AN are positively correlated with exercise.

3.2. Methods

3.2.1. Design

This study was carried out from December 2000 to February 2001 in the city of Buenos Aires. Blood cholesterol measurements documented at diagnosis were recorded from the clinical notes of patients diagnosed with AN by the criteria of the American Psychiatric Association's Diagnostic Manual (DSM IV 1994) at the

Asociación lucha contra la Bulimia y la Anorexia (ALUBA) (Association Against Bulimia and Anorexia). An unpaired comparison was carried out with data of the general Argentinean public matched for the patients' sex and age. The sources of the Argentinean data were two published studies (Selles et al 1997; Coniglio et al 1997). In patients, documented cholesterol concentrations at diagnosis were compared with follow-up and correlated with the age of the patients, BMI, occurrence of vomiting, exercise and tobacco smoking.

3.2.2.2. Sample

Patient group

Sample size was calculated for total cholesterol on the basis of the data in seventeen published studies, which looked at cholesterol in patients with AN and in controls. From these studies, it was calculated that the weighted mean cholesterol value of patients with AN exceeded that of the control subjects by 13%, the mean difference being 27.5 mg. The 'Sample Size Calculator' programme (http://www.ucalgary.ca) indicated that to achieve 95% power a sample size of 170 subjects in each group was required. Therefore, 170 or more clinical notes were planned for review and compared with a minimum of 170 controls.

Inclusion criteria

- The most recent clinical notes of patients admitted to the ALUBA with diagnosis of AN (DSM IV criteria) containing data of blood total cholesterol concentrations.

The clinical notes with the following characteristics were excluded:

- Documented psychiatric illness other than AN
- Male sex
- Pregnancy

methods in the same laboratory, the quality control of which is supervised by the Argentinean Biochemistry Foundation.

Exclusion criteria

- Male sex
- Pregnancy
- Diabetes, altered thyroid function, renal or hepatic diseases (subjects who reported suffering from any of the above mentioned diseases were excluded from the studies).

3.2.3. Procedure

Clinical notes were hand-searched from the most recent admission retrospectively and those which met the patient criteria were selected. Data (blood total cholesterol, age, occurrence of vomiting, exercise, weight and height, use of tobacco and other biochemical data) were entered into a spreadsheet. Raw data from the Argentinean studies were then transferred into a spreadsheet and compared with the patient data. BMI was calculated from the body weight and height of patients on admission, but not always at exactly the same time as the blood determinations were carried out. However, most of the clinical notes had cholesterol checked very close to admission date.

When reviewing the clinical notes, it was observed that many of them had a second TC determination. This information was recorded and compared with the levels of TC on admission. Blood fasting glucose and T3 and T4 levels were also recalled to rate the prevalence of hyperthyroidism and diabetes in the patient group, since these may be confounding variables in the analysis of blood cholesterol

The variables of the lipid profile in patients were compared with reference values. Cholesterol concentrations differ significantly between adolescents and adults, therefore there are different international recommendations according to the age group: the NCEP (1992) for subjects younger than 20 years and the NCEP (2001) for subjects between 20 and 29 years. There is not one source that gives desirable values

for both age groups and therefore the values for these two recommendations were averaged. Because there is no specific cut-off point for desirable HDL concentrations in the NCEP (1992) recommendation, it was decided arbitrarily to take the value of the 25 centile for normal HDL distribution: 42 mg/dl. Cut off points of the NCEP for Children and Adolescents (1992) and for adults (2001) are the following: TC: ≥ 200 mg/dl; ≥ 240 mg/dl, LDL: ≥ 130 mg/dl; ≥ 160 mg/dl, HDL: ≤ 42 mg/dl; ≤ 45 mg/dl and TG: 150 mg/dl; ≥ 150 mg/dl.

Ethical approval was obtained from King's College London (99/00-91).

3.2.4. Statistics

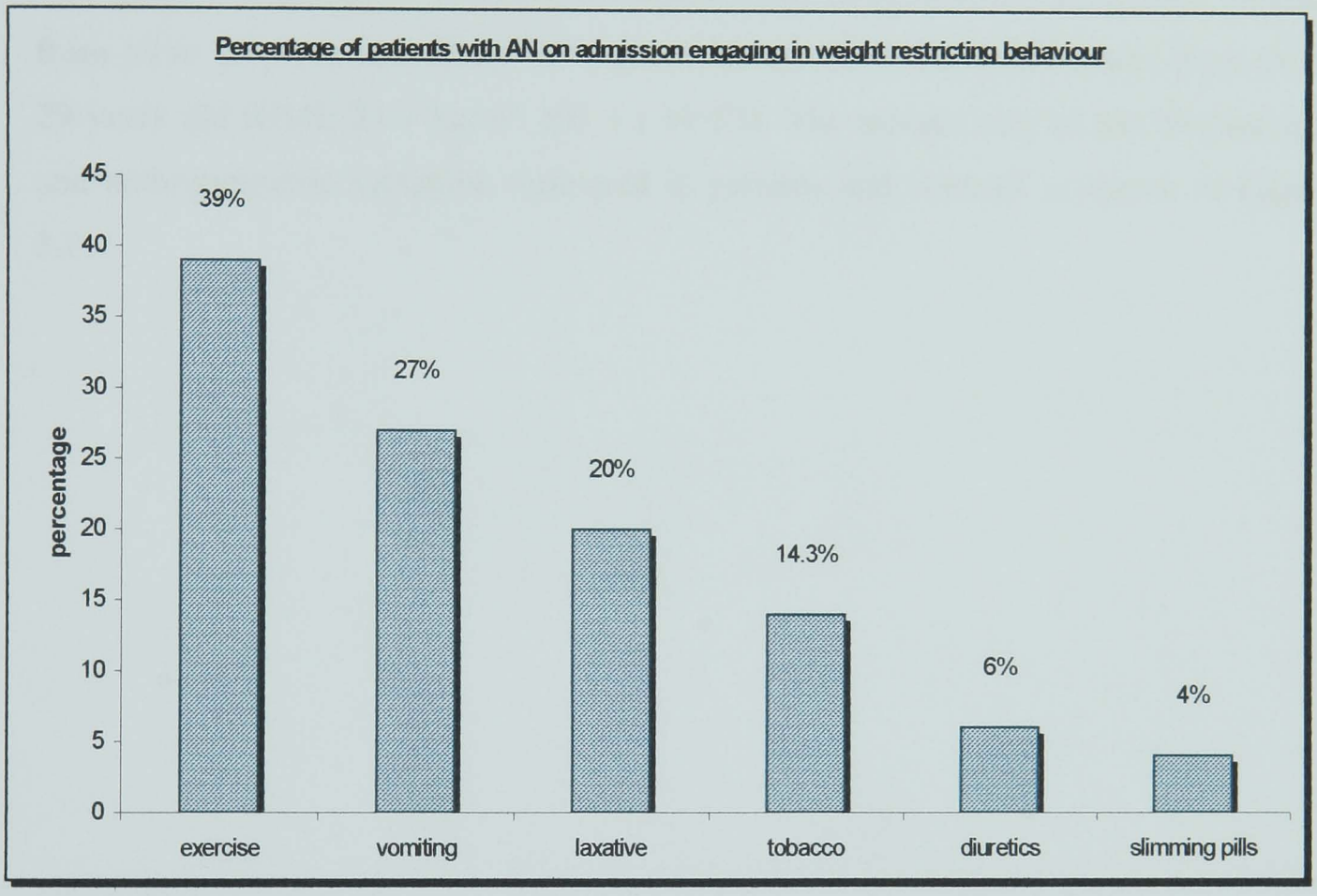
The two sets of raw data (1. Data from patients and 2. Data from healthy Argentinean population) were combined in SPSS. Descriptive statistics were produced to describe the samples and test for normality. Blood TC concentrations were compared between patients and control subjects by the two-tail unpaired T-test. The paired T-test was used to compared the admission and follow up concentration (second determination) of TC in patients. Differences were regarded as statistically significant if $P< 0.05$. The Univariate General Linear Model test was used to adjust blood total cholesterol concentrations for other variables such as BMI and age. The Pearson Chi^2 method was used to compare cholesterol values with international normality ranges (National Cholesterol Education Program (NCEP) 1992 and 2001 cut-off points). In the patient group, the Pearson's correlation test was used to associate variables.

Declaration

The author of this thesis designed this study, carried out the fieldwork and analysed the data.

The majority of the patients (62% N=191) were restricting type (patients who do not regularly engage in binge-eating or purging) and 117 patients (38%) non-restricting type (patients who use binge-eating or purging), according to the DSM V (1994) criteria. The percentage of patients engaging in weight restricting behaviour such as exercised, vomited, used laxatives, etc. is shown in Graph 3. 1.

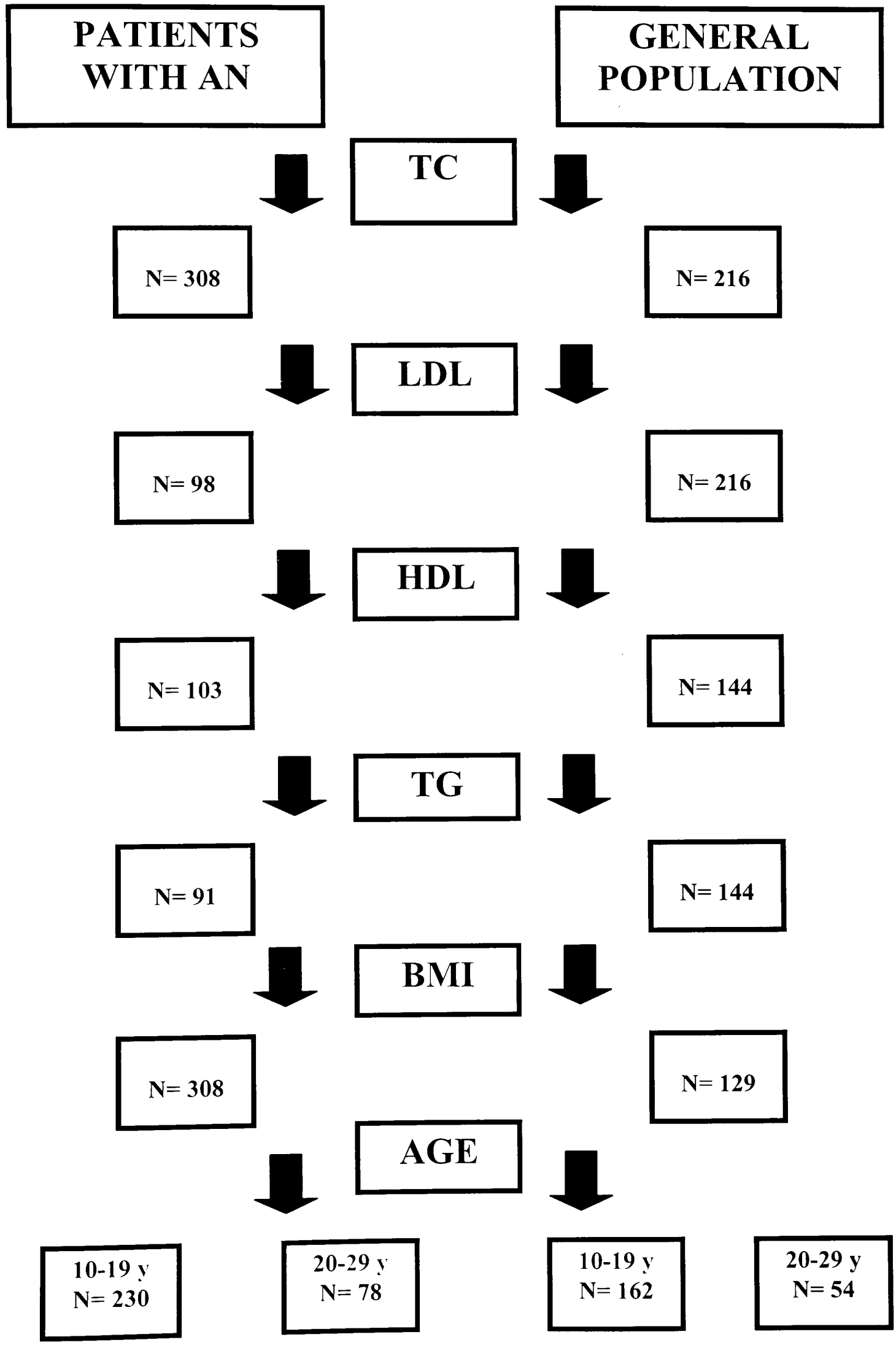
Graph 3.1



No significant correlations were found between blood TC levels and the age of the patients, BMI, occurrence of vomiting or tobacco and between HDL concentrations and exercise, therefore the hypotheses that these variables were related to blood TC concentrations were not proved.

193 patients had data of a second total cholesterol determination during treatment in their clinical notes. The mean time between the first and second determination of total cholesterol was 9.8 months (SD 9.9). Total cholesterol concentrations were significantly higher (p= 0.011) in the first (mean: 197.1 mg/dl, SD 43.5) than the second determination (mean: 186.3 mg/dl, SD 38.9). However, no

Figure 3.1: Sample size (N) of the variables compared in patients and in the general population



The patient group had a significantly lower BMI than the general population group (Table 3.1). Significantly higher concentrations of total cholesterol, LDL and HDL were found in patients compared with subjects from the general population group, as was hypothesised. Hypercholesterolemia and high LDL concentrations in the younger patients persisted even when these were adjusted for BMI and age. However, when HDL and the ratio of triglycerides/HDL (TG/HDL) were adjusted for age and BMI the differences were non-significant ($p > 0.050$) (Table 3.2).

Table 3.1: Biochemical and other variables in patients with AN on admission and in the general healthy population

Variables	Patients with AN			General population			P values*
	N	Mean	SD	N	Mean	SD	
Age (years)	308	17.3	3.8	216	18.1	4.7	NS
BMI (kg/m^2)	308	17	2.0	129	20	3.2	< 0.00
TC (mg/dl)	308	191.4	41.3	216	169.2	34.2	< 0.00
HDL (mg/dl)	103	59.3	16.0	144	49.3	10.9	< 0.00
LDL (mg/dl)	98	119.6	42.6	216	95.3	32.5	< 0.00
TG (mg/dl)	91	81.4	41.3	144	88.2	41.3	NS
TC/HDL	103	3.6	1.2	144	3.6	0.8	NS
LDL/HDL	90	2.1	0.9	144	2.1	0.8	NS
TG/HDL	71	1.3	0.8	144	1.9	1.1	< 0.00

*T-test

N= number of subjects

NS=non-significant

TC=total cholesterol

TG=triglycerides

Conversion factor: 1mg/dl cholesterol= 0.0259 mmol/l

Among subjects younger than 20, there was a significantly greater number of patients than controls having TC and LDL concentrations above the cut-off points (Table 3.3). The same pattern was found in subjects aged from 20 to 29 years, however differences in LDL were not significant. Notably HDL values were higher in patients than controls in both age groups.

3.4. Discussion

TC and LDL concentrations were significantly higher in patients than in subjects from the general population. These findings accord with the studies of Mordasini and co-workers (1978), which first described type II hypercholesterolemia (high total cholesterol and LDL) in a group of patients with AN. Hypercholesterolemia II is one of the most harmful types, having the propensity to cause morbidity and mortality at a young age (NCEP 2001).

The present study demonstrates that cholesterol concentrations were not associated with BMI, vomiting, exercise or tobacco use. This implies that there may be characteristics of AN, other than slimness, purging behaviour or tobacco, which account for the elevation of cholesterol. However, the non-significant associations may be due to a small number of subjects in these categories; only a small number of patients vomited, smoked or exercised. Tobacco is often consumed by non-restricting type of patients with AN, who use vomiting, laxatives, diuretics or slimming pills to lose weight. These patients are less socially inhibited and have more impulsive personalities (Garfinkel et al 1980) that may lead them to smoke. In the present study, only a minority of patients (38%) were of this type, which may explain the lack of association. Besides, the amount and frequency of tobacco smoking, which affect blood cholesterol values, were not reported in the clinical notes. The lack of association between age and TC may be due to the exclusion of older patients.

Blendis and Crisp (1968) found higher cholesterol concentrations in patients who vomited (N=5) than in those who restricted food intake and abstained from vomiting (N=3). Case and colleagues (1999) observed that cholesterol values were higher in patients with AN who purged (N=9) compared with patients with bulimia

of cholesterol normalise. Desirable cut-off points for the lipid profile components are clearly required for adolescents and young adults. There is also a lack of data on desirable cholesterol values for the Argentinean population.

3.5. Limitations of this study

Some important limitations, which could have made the comparisons between patients and controls less accurate, need to be mentioned:

1) The biochemical data from the general population studies and from the clinical notes of the patients were determined in different laboratories and they may have used different methods. However, it is worth noting that half of the patients' lipid profile analyses were carried out in the lab of the Gastroenterology Hospital.

2) LDL concentrations in the controls were calculated, while it was not always possible to known whether they were measured or calculated in patients

3) It is uncertain whether blood was collected in the fasting state in all patients. However, the protocol of ALUBA states blood to be taken in fasting.

4) It is possible that those with blood lipid measured may have been at higher risk for a lipid problem. However, this is unlikely because the protocol of ALUBA is to determine cholesterol levels in all the patients on admission.

5) Physical activity is a difficult variable to assess. In patients, these data were taken from a questionnaire where patients reported whether they performed exercise and therefore the validity is questionable.

6) Body weight was measured in patients, while in the general population, body weight was in some subjects measured and in others reported.

3.6. Conclusion

The present study demonstrated that total cholesterol, LDL and HDL concentrations were higher in patients with AN compared with a healthy population. The elevated total cholesterol concentrations on admission decreased at follow up, which was after an average of nine months of treatment. The abnormal lipid profile places patients at risk for future cardiovascular disease. Untreated AN may be a

contributing factor for the elevated cardiovascular mortality among young Argentinean females.

This study justifies the need for the next study, which assessed cholesterol concentrations and investigated their possible associations in a selected clinical group of women with AN. Further studies investigating the causes and the outcomes of the hypercholesterolemia in patients with AN are needed.

Chapter 4 - Follow up of patients with AN

4.1. Introduction

There is existing evidence to suggest that some risk factors for cardiovascular diseases are higher in patients with AN than in the general population (Chapter 1 and chapter 3). Hypercholesterolemia is one of these factors, although it is uncertain whether elevated cholesterol levels are confined to the period of under-nutrition or remain following treatment.

In addition to the high cholesterol levels, other biochemical abnormalities reported in AN that are potentially associated with cardiovascular disease include: hypercortisolemia (Tamai et al 1991 and Zumoff et al 1983), low blood glucose levels (Zuniga-Guajardo et al 1986) and low essential fatty acids (Holman et al 1994) and low oestrogen values (Feillet et al 2000). Low (Langan and Farrell 1985) and normal (Mira et al 1989 and Van Binsbergen et al 1988) blood levels of the antioxidant Vitamin E have been reported. Behavioural and dietary risk factors for cardiovascular disease in AN include the use of tobacco (Haug et al 2001), low energy intake (Beaumont et al 1981), low intake of the cardio-protective vitamins (i.e. Vitamin B12, B6, folate, vitamin A) (Philipp et al 1988 and Vaisman et al 1992), and increased consumption of caffeine drinks (Sours 1983 and Forman et al 1997). However, other findings such as the high concentrations of carotene (Boland et al 2001) are associated with lower rather than higher risk.

Controversy among the above studies may be explained by some methodological problems. They include insufficient sample sizes, lack of control groups, lack of attention to confounding factors (i.e. tobacco smoking, meal frequency, vomiting behaviour, etc.), and large variability in some of the parameters such as blood cholesterol. There are inconsistencies in the use of the diagnostic criteria and many studies do not discriminate between patients with restricting and non-restricting types of AN. Well controlled studies taking these factors into account are clearly needed.

The impact of treatment and recovery on these risk factors for cardiovascular diseases is a controversial arena. Some studies have concluded that the high levels of cholesterol return to normal after treatment (Blendis and Crisp 1968), while others have not found any difference in cholesterol values between patients at admission and at discharge of treatment (Halmi and Falk 1981). Levels of blood glucose and hormones usually return to normal after treatment. Vitamin E blood levels increase after re-feeding (Vaisman et al 1992) and carotene concentrations usually decrease (Casper et al 1980). There is no data on homocysteine and fatty acid levels after treatment.

Methodological problems also exist in relation to these studies. They include insufficient sample sizes, short follow-up periods and loss of subjects at the follow-ups. Some studies have failed to specify the length of time subjects have been followed up. The extent of recovery is also sometimes poorly and inconsistently defined. The measurement of recovery tends to be based on normal BMI rather than on attempts to measure psychological recovery, or absence of purging behaviours. Patients on admission, and those who had been undertaking treatment, are generally studied together. Many studies do not always have adequate control subjects and do not generally assess the other components of the lipid profile (HDL, LDL and triglycerides). Recovered patients are rarely followed up on longitudinal studies.

This study aimed to compare biochemical parameters related to cardiovascular risk in a group of patients with AN with sex and age-matched healthy control subjects, and to follow up patients after treatment. For this purpose the chapter was divided into two separate studies:

- ***Study 3.*** Comparison of cardiovascular risk factors, hormonal status and nutritional indices in patients with AN on admission and controls.

- ***Study 4.*** Comparison of cardiovascular risk factors, hormonal status and nutritional indices before and after treatment in patients with AN.

4.2. -Study 3- Comparison of cardiovascular risk factors, hormonal status and nutritional indices in patients with AN on admission and controls

4.2.1. Hypotheses

Risk factors for cardiovascular disease were expected to differ between patients with AN and healthy controls. The hypotheses tested in this study were the following:

Main hypothesis

Patients with AN on diagnosis have: a) higher blood concentrations of TC, LDL, Apo B, triglyceride, cortisol, homocysteine and fibrinogen, and b) lower concentrations of glucose, essential fatty acids and lipid-antioxidant vitamins (tocopherol and retinol) than controls

Secondary hypothesis

High TC and LDL concentrations in patients with AN on admission are negatively correlated with estradiol and thyroid hormones and positively correlated with insulin and cortisol concentrations.

4.2.2. Methods

4.2.2.1. Design

This was a case-control study. Patients with AN (DSM IV criteria 1994) attending the ALUBA in Buenos Aires were recruited on admission. The control group, matched for sex, age and social class, was selected from the general public.

4.2.2.2. Sample

Sample size calculation method was detailed in Chapter 3 and it was based on the calculations for blood cholesterol. However, the size of the sample of patients in this study was restricted to the number of patients that the ALUBA permitted for this study and the allotted time to carry out the fieldwork.

Patient sample

Inclusion criteria

Thirty restricting and non-restricting type women with AN (DSM IV 1994) were recruited on admission to the ALUBA.

Aged 15 years or older

Exclusion criteria

Male sex

Pregnancy

Bulimia Nervosa (BN) or any psychiatric illness other than AN

Control subjects

Inclusion criteria

Thirty normal-weight control subjects recruited from the University of Buenos Aires, radio, Internet and community centres.

Matched by sex, age and social class (estimated by father's occupation) with the patient group.

Exclusion criteria

Abnormal BMI (greater than 25kg/m^2 or less than 18.5 kg/m^2)

Male sex

Pregnancy

Any diagnosed illness

History or current diagnosis of AN or BN by the DSM IV

Menstrual irregularities

Use of strict diets

Use of diuretics, laxatives, slimming pills, binge eating or vomiting.

4.2.2.3. Procedure

Patients on admission to ALUBA, who met the patient criteria, were informed about this study and invited to take part in it. Control subjects were recruited via advertisements. Lifestyle and background data from the patients were obtained from the institution's clinical record and from a questionnaire that all patients completed on admission (Appendix 4.1). This questionnaire included information about the patient's family history of chronic diseases. The same questionnaire was adapted, including some variables to exclude those with eating problems, and issued to control subjects (Appendix 4.2).

Patients and controls were given information about the study and signed a written consent form agreeing to participate in the study (Appendices 4.3, 4.4 and 4.5). Parental consent was obtained, in addition to individual consent, in those subjects under 18. Ethical approval was obtained from King's College London (99/00-91).

Patients and controls were required to come to hospital, having fasted for nine hours, for a one-hour appointment. A phlebotomist took twenty millilitres of fasting blood in the morning at between 8 to 9.30 am to avoid the normal decline of cortisol during the day. Body weight, height and blood pressure were measured. Patients were evaluated on two occasions: 1. Admission and 2. Follow up. The admission data are presented in this study and the follow up data will be shown in the next study (Study 4). The control subjects were also evaluated on two separate occasions and the measurements averaged in order to obtain more accurate information. The second measurement in the control group was taken at a mean time of 27.8 days (SD 24.1) after the first measurement. The difference between repeated measurements, for example for blood TC, was 6 mg/dl, which was not significant.

The results of the study were communicated by mail to each control subject (advising them to seek medical consultation in the case of abnormal results) and also to the consultant in charge of the patients.

Biochemical analyses

Blood for lipids, cortisol, glucose, homocysteine and hormones was collected in a silicone vacutainer containing no anti-coagulant to obtain serum. Blood for fatty acids, Apo A and Apo B, antioxidant vitamins and fibrinogen was collected into EDTA tubes to obtain plasma.

Plasma and serum were separated in a Gelec 75 centrifuge at 3000 rpm per gram for 15 minutes. Blood samples were separed into aliquots, labelled and stored at -17°C in cryo-vials within two hours of the blood being taken. Blood samples for antioxidant vitamins, fatty acids, homocysteine and hormones were kept at –70°C and transported to London, where they were analysed about 1.5 years later. All the other parameters were tested within the month of blood collection.

The lipid profile (TC (cv: 2%), LDL, HDL (cv: 3%), and TG (cv: 2%)), cortisol (cv: 5%) and glucose (cv: 1.5%) levels were analysed in the Centralab (Buenos Aires). The Centralab is subjected to quality control by the Argentinean National Administration of Medicine, Technology and Food (ANMAT). LDL cholesterol concentrations were calculated according to the Friedwald formula (1972), which is only valid if TG levels are lower than 200 mg/dl. The lipid profile and glucose levels were determined by enzymatic colorimetric endpoint method with a BM/Hitachi 917, and the cortisol by chemiluminiscence immunoassay with a DPC Immulite autoanalyser. Plasma fibrinogen (cv: 5%), Apo A and Apo B levels were analysed in the Faculty of Pharmacy and Biochemistry, Buenos Aires University, by radial quantitative immunodiffusion (Diffuplate Biocientifica S.A.). Free T3 (cv: 5%), T4 (cv: 5%), insulin (cv: 5%) and homocysteine (cv: 2.5%) were analysed in the Department of Chemical Pathology, St. Thomas' Hospital London. Plasma homocysteine was measured by the SCIEX API 2000 Tandem Mass Spectrometry, serum insulin by the DPC Immulite Automated Immunoassay Analyser, and Free T3

and Free T4 and oestradiol were analysed in serum by the ADVIA Centaur immunoassay using direct chemiluminescent technology.

Blood samples for tocopherol, retinol, carotene (cv: 5%) and fatty acids (cv: 4%) were protected from light throughout processing and handling. These analyses were completed in the Department Nutrition and Dietetics of King's College, London. The methodology is detailed in the appendix 3.6.

Anthropometric measurements

1) Height (metres) was measured with subjects standing, back to a portable 2-meter stadiometer (Dolz, Model DPP). The head was adjusted horizontally on the Frankfurt plane (the lower borders of the eye sockets in the same horizontal plane as the external auditory meati). Bare feet were kept parallel, with the heels together. The measurement was taken after gentle expiration.

2) Weight (kilograms) was measured in bare feet with minimal clothing. A digital personal weighing scale was used.

Weight and height were used to calculate BMI and the results compared between patients and controls. A value between 20 and 25 kg/m^2 is accepted as normal for adults. A value below 20 kg/m^2 is defined as low body weight, between 25 and 30 kg/m^2 as overweight and above 30 kg/m^2 as obesity. For children and adolescents there are centiles of weight and height for age based in the normal distribution in the Argentinean population (Chapter 2).

Blood pressure

Seated systolic and diastolic blood pressure were recorded in the right arm in duplicate by the same trained observer. Patients and controls were asked to sit relaxed for 10 minutes before the measurement been taken. A mercury sphygmomanometer with an appropriate cuff (Kosan, Japan) was used to measure blood pressure. Diastolic blood pressure was recorded at Korotkoff phase IV.

4.2.3. Results

4.2.3.1. Description of the treatment for patients with AN

Aluba is a non-governmental organisation founded by Dr. Mabel Bello in 1985 and is currently the chief referral centre for eating disorders in Argentina. The main institution is located in the Gastroenterology Hospital of Buenos Aires and has forty branches around Argentina; two in Uruguay and one in Spain. Aluba treats patients with eating disorders (AN, BN, Non-Specified Eating Disorder and Binge Eating). The staff includes psychologists (two to every 60 patients), clinicians, nurses and psychiatrists. The treatment involves nutritional, psychological and psychiatric therapy, including the use of drugs such as antidepressants (for example: amitryptiline), tranquillisers (for example: benzodiazepines), and anti-psychotics (for example: chlorpromazine). Iron supplements are prescribed to patients routinely but multivitamins are rarely given, because they believe that weight restoration corrects any nutritional deficiency. None of the subjects was taking vitamin supplements at the time of the investigation. The total length of treatment is usually four to five years.

On admission to treatment, patients have an interview with a psychiatrist and a psychologist. After this, they are referred, according to the seriousness of the illness, either to the **pre-admission module** or the **day hospital**.

The **pre-admission module** consists of a month of psycho-educational group meetings, where patients are assigned to hospital three times a week for two hours. This module is designed so that patients can understand the modality of the treatment and also to facilitate a better diagnosis of the case. After this initial month, patients are referred either to the day hospital or to outpatient treatment, depending on the psychopathology and health situation. In the **day hospital** patients attend hospital from 8 a.m. to 5 p.m. and are located in different rooms by age groups. There is also an intensive care room for the most serious cases, offering constant medical supervision. 'Leaders' are patients in a more advanced stage of treatment, who are in charge of supervising the rest of the patients' meals and behaviour. They report any abnormal behaviour of the patients to the rest of the group and to the psychologist

responsible for the group. During the hospital day, patients have three hours of psycho-educational group meetings and two hours of art therapy. Patients usually see a clinician every month, depending on the seriousness of the case, and blood testing is undertaken approximately every three months.

A nurse weighs the patients every morning but they are kept in ignorance of their weight. Food intake is monitored by regularly checking patient weight gain. Patients are given six daily meals in the hospital, including breakfast (lasting half an hour), snack 1, lunch (lasting one hour), snack 2 and tea. Dinner is eaten at home. They can either bring their own food to the hospital or buy it in the hospital kitchen. The hospital kitchen agrees the menu with the clinician. They also offer menus for different dietary plans (hypo-caloric, normal, hyper-caloric 1 and 2, hepato-protector, gastro-protector, low in fibre, high in fibre, rich in iron, rich in fibre, low in fat, etc.). Aluba does not work with dietitians and there is no protocol for the calculation of energy requirements or dietetic goals. The clinician prescribes the dietary plan and psychologists and group leaders supervise the quality and quantity of food eaten. Supervision of meals is based on 'common sense'. That is, a food serving should include a full plate containing varied foods (for example: a piece of meat, a portion of food rich in starch and vegetables), a drink and a pudding. Patients are pressured to eat by their peers and in extreme cases by psychologists. Everyone is expected to consume the food they are given within the allotted time for that meal. After each meal all the patients are required to remain in the same room under supervision for one hour of relaxation and in this time no-one is allowed to visit the toilet. This is to avoid the use of purging methods to lose weight. Patients have to keep a food diary where they write down every portion of food and drink consumed daily. However, this information could not be analysed because it was qualitative not quantitative. Parents are asked to recall in this diary any comment on the patient's diet, mood, behaviour and on the use of the toilet.

Patients are not allowed to have anything to do with the preparation of food or to express opinions about food, to be vegetarians, to be alone, to handle money, to study, to work, to smoke, to consume drugs, or to drink alcohol until they show symptoms of recovery. They only exercise under supervision when BMI is within

normal values. The family is in charge of controlling the use of the toilet, meals at home, sleeping hours, socialising activities, etc. Freedom in these activities is given back to the patients when they recover weight and show some psychological improvement. These privileges are lost when patients are not following the rules of treatment. The parents have fortnightly group meetings with a psychiatrist, where treatment is discussed and doubts are resolved. There are also special group therapy sessions for brothers, sisters, and friends, for conflicted families and for patients with an associated pathology such as a personality disorder (for example: borderline or antisocial behaviour).

There is another modality of treatment called **out-patient treatment,** comprising two-hour psycho-educational group settings three times a week. This treatment is considered appropriate for patients who are more advanced in treatment, for those whose illnesses are less severe, and for patients who, because of family or economic reasons, cannot attend the day hospital treatment.

4.2.3.2. Characteristics of the subjects

The sample comprised thirty female patients with AN recruited within a month of admission at ALUBA, between October 2000 and June 2001. Patients were recruited from the **day hospital** and **pre-admission module**. The average age at the onset of the illness, which was considered as the onset of dieting, was 17.2 years old (SD 3.6). Mean duration of the illness was 6.3 years (SD 6.5) and it was calculated as the difference between current age and age at onset of AN. The control group included thirty healthy subjects. Half the participants were from a middle socio-economic class and the other half a lower socio-economic class. Table 4.1 shows some characteristics of patients and control subjects.

Table 4.1: Characteristics of patients on admission and controls (Mean and SD)

Variables	Patients with AN					Control subjects					
	N	Mean	SD	Medium	Range	N	Mean	SD	Medium	Range	Val
Weight (kg)	30	43.6	5.5	44.0	29.0-50.0	30	54.3	7.1	50.4	40.0-70.0	
Height (m)	30	1.57	0.1	1.57	1.37-1.73	30	1.60	0.1	1.62	1.44-1.76	
BMI (kg/m2) *	30	17.0	2.0	17.0	13.0-19.0	30	21.0	2.1	20. 0	19.0. -25.0	0.0
Age (years)	30	23.5	8.7	20.5	15.0-53.0	30	23.6	5.1	23.0	15.0-32. 0	
Age at menarche (years)	27	13.4	1.8	13.0	10.0-17.0	30	13.0	1.4	13.0	10.0-16.0	
Age at onset (years)	27 (1)	17.2	6.4	16.0	13.0-46.0	N/A	N/A	N/A	N/A	N/A	
Duration of the illness	27 (1)	6.3	6.5	6.0	1.0-34.0	N/A	N/A	N/A	N/A	N/A	
Meal frequency (number meals/day)	22 (1)	4.1	1.9	4.0	0.5-8.0	30	4.3	1.5	4.0	2.0-8.0	
Maximum weight ever (kg)	30	52.3	6.0	52.0	41.0-60.0	30	57.9	5.1	57.0	45.0-69.0	
Minimum weight ever (kg) *	29 (1)	38.7	6.2	40.0	24.0-47.0	30	51.4	8.0	50.0	40.0-85.0	0.0
Systolic blood pressure (mmHg)*	30	97.5	10.5	100.0	86.6-120.0	30	105.4	7.1	105.0	94.1-120.0	0.0
Diastolic blood pressure (mmHg)	30	68.4	9.7	70.0	53.3-90.0	30	71.1	8.7	72.5	58.0-90.0	

T-test *p: <0.05

(1) Missing information from clinical notes

Not all the patients with AN met the DSM IV criteria for AN in terms of fulfilling weight and amenorrhea criteria requirements. This is because many patients had been undertaking treatment prior to the investigation, and weight gain had occurred.

BMI, systolic blood pressure and minimum weight ever achieved were significantly lower in patients compared with controls.

On admission, 57% patients (N=30) had amenorrhea (lack of menstrual bleeding during the past three months), 20% oligomenorrhea (irregular menstrual bleeding), and 23% were menstruating.

There were eighteen patients (60%) with restricting type AN (patients who do not regularly engage in binge-eating or purging behaviour, for example: self-induced vomiting or misuse of laxatives, diuretics or enemas), and twelve patients (40%) with non-restricting type. Approximately 30% of patients vomited, 17% used laxatives, 10% homeopathic slimming pills and 3.5% diuretics. Smoking tobacco involved 17% of patients, compared with 36% of controls.

The results of patients and control subjects in terms of family history of cardiovascular disease related illnesses are compared in Table 4.2. A substantially greater percentage of patients, when compared with control subjects, declared having a family member who suffered from obesity, high cholesterol levels, hypertension and depression. However, eating disorders were the only significantly more frequent diseases in the patients' families compared with the controls.

Table 4.2: Percentage of patients on admission and controls with a family history of cardiovascular related illness (including 1st and 2nd degree relatives).

Cardiovascular related disease	Patients (N= 30) % (N)	Controls (N= 30) % (N)
Hypertension	50 (15)	38 (13)
Obesity	33 (10)	10 (3)
Diabetes	30 (9)	27 (8)
High Cholesterol levels	40 (12)	27 (8)
Atherosclerosis	17 (5)	13 (4)
Depression	43 (13)	20 (6)
Eating disorders	20 (6)*	0

* p: 0.024 Fisher's Exact Test

The term atherosclerosis was understood by most of the control subjects. In cases where this was not clear, it was defined as the blockage in the passage of blood through the arteries leading to chest pain, angina, heart problems, heart attack and stroke.

Table 4.4 shows a comparison in the TC and LDL levels of patients by age group with reference values. Those patients older than 20 years showed higher cholesterol levels than patients 20 years old or younger. The mean total cholesterol value in the group of older patients was above desirable levels. 47% of older patients had TC levels above desirable values.

Table 4.4: Total cholesterol, LDL concentrations and desirable lipid concentrations in patients aged 20 years or younger and in patients older than 20 years

Variables	Patients 20 years or younger N=15		Patients older than 20 years N=15	
	Mean	SD	Mean	SD
TC (mg/dl)	166.9	35.6	215.8	55.6
LDL (mg/dl)	98.2	28.8	135.5	45.9
Desirable TC values (mg/dl)	<170.0 (*1)		<200.0 (*2)	
Desirable LDL values (mg/dl)	<110.0 (*1)		<160.0 (*2)	

(*1) Report of the expert panel on blood cholesterol levels in children and adolescents, 1992.

(*2) National Cholesterol Education Program, 2001.

Hormones

Table 4.5 shows that free T3, the T4/T3 ratio and estradiol values were significantly higher in patients compared with controls after adjusting for age and BMI (kg/m^2). The relevance of measuring of the T4/T3 ratio is that in hypothyroidism the ratio T4/T3 decreases, whereas in AN free T3 decreases more than T4 and therefore the ratio is elevated. Homocysteine concentrations did not differ between patients and controls. Homocysteine levels increased with age but not significantly; the mean homocysteine concentration in patients aged 20 or younger being 9.1 µmol/l (SD 2.1, N=15) and in older patients 11.0 µmol/l (SD 5.14, N=15).

Lipid-soluble vitamins

Tocopherol levels differed between patients and controls after adjusting for TC (Table 4.6). The patient mean tocopherol level fell within the reference value of British women aged between 18 and 45 years of 23.7 μmol/l (Gregory et. al 1990). A plasma tocopherol level of 0.5 mg/dl (11.6 mmol/l) is an indicator of biochemical deficiency and the patients were considerably above this level (Horwitt 1980).

Retinol concentrations in the patients were significantly lower than in controls (p: 0.010) and these were also below the reference value of British women aged between 18 and 45 years of 1.8 μmol/l, SD 0.03 range= 0.10-2.10 (Gregory, et al 1990). A plasma retinol concentration below 20 μg/100 ml (0.7 μmol/l) indicates vitamin deficiency and subjects usually exhibit deficiency signs. Only one of the patients was below this level. Interestingly, (p: 0.003 r= -0.376) and carotene (p: 0.001, r= -0.429) levels in patients correlated negatively with BMI.

carotene levels in patients were higher than controls, although this difference was not significant after adjusting for total cholesterol values. The mean carotene level in controls was slightly higher than 0.33 μmol/l, SD 0.01, range= 0.10-0.70 which is the mean reference value for British women aged between 18 and 45 years (Gregory et al 1990). There were significant differences in the concentrations of other plant antioxidants such as cryptoxanthine between patients and controls. Moreover, total carotenoids (and carotene, crytoxanthine and lycopene) were significantly higher in patients compared with controls.

Table 4.7: Fatty acid proportions and concentrations of plasma total lipids in patients on admission and controls (Mean and SD)

Fatty acid profile	Patients with AN				Controls				Holman, et al (1979) USA females	P value	
	Mean		SD		Mean		SD				
	%	mmol/l	%	mmol/l	%	mmol/l	%	mmol/l	%	%	Mmol/l
Palmitic acid (16: 0)	**19.25**	**2.17**	1.98	1.06	**31.80**	**1.89**	72.15	0.38	20.63	N/S	N/S
Palmitoleic acid (16: 1 n-7)	**2.30**	**0.29**	1.20	0.29	**4.04**	**0.19**	12.01	0.07	3.29	N/S	N/S
Stearic acid (18: 0)	**7.58**	**0.74**	0.79	0.21	**12.54**	**0.67**	28.62	0.12	7.31	N/S	N/S
Oleic acid (18: 1 n-9)	**18.15**	**2.07**	2.72	1.19	**31.14**	**1.73**	73.38	0.51	22.75	N/S	N/S
Linoleic acid (18: 2 n-6)	**31.99**	**3.32**	4.70	1.01	**32.76**	**3.08**	4.29	0.76	27.68	N/S	N/S
Gamma linolenic acid (18: 3 n-6)	**0.13**	**0.01**	0.10	0.01	**0.12**	**0.03**	0.05	0.01	0.52	N/S	0.000
Alpha linolenic acid (18: 3 n-3)	**0.36**	**0.03**	0.12	0.03	**0.32**	**0.02**	0.11	0.01	0.63	N/S	N/S
Mead acid (20: 3 n-9)	**0.15**	**0.03**	0.04	0.02	**0.14**	**0.01**	0.03	0.00	0.65	N/S	0.003
Dihomogamma linolenic acid (20:3n-6)	**1.70**	**0.11**	0.48	0.09	**1.66**	**0.46**	0.40	0.24	1.96	N/S	0.000
Arachidonic acid (20: 4 n-6)	**6.79**	**0.47**	1.28	0.27	**6.90**	**0.60**	1.27	0.16	7.61	N/S	N/S
EPA (20: 5 n-3)	**0.53**	**0.28**	0.47	0.35	**0.34**	**0.03**	0.14	0.02	0.71	0.001	0.001
7,10,13,16-Docosatetraenoic (22: 4 n-6)	**0.62**	**0.05**	0.09	0.02	**0.59**	**0.05**	0.12	0.01	0.33	N/S	N/S
4,7,10,13,16-Docosapentaenoic (22: 5 n-6)	**0.57**	**0.04**	0.13	0.01	**0.53**	**0.04**	0.13	0.01	1.03	N/S	0.005
DPA (22: 5 n-3)	**0.46**	**0.05**	0.17	0.01	**0.40**	**0.03**	0.11	0.01	0.47	N/S	0.010
DHA (22: 6 n-3)	**1.51**	**0.04**	0.73	0.01	**1.47**	**0.11**	0.36	0.03	1.57	N/S	0.000
Total SFA	**29.13**	**3.19**	2.88	1.54	**48.38**	**4.39**	112.76	0.89	45.50	N/S	0.001
Total PUFA	**44.81**	**4.29**	4.97	1.15	**45.22**	**4.51**	5.55	0.91	44.40	N/S	N/S
Ratio Mead/Arachidonic (20: 3 n-9/20: 4 n-6)	**0.02**	**0.02**	0.01	0.13	**0.02**	**0.02**	0.00	0.00	0.10	N/S	0.000
Total n-3	**2.87**	**0.24**	1.25	0.09	**2.52**	**0.20**	0.23	0.05	3.94	0.000	N/S
Total n-6	**41.79**	**4.03**	4.82	1.12	**42.56**	**4.31**	5.22	0.88	39.47	N/S	N/S
Ratio n-6/n-3	**16.26**	**17.96**	4.89	5.41	**17.51**	**21.80**	3.50	4.43	10.0	0.000	0.004

T-test

Comparison between patients and controls

Significant <0.01

obtained from an evaluation sheet, which a psychiatrist routinely recorded approximately every three months.

4.3.3. Statistics

The t-test for related samples was used for testing repeated measurements in the patient group after a 4-month treatment period.

4.3.4. Results

Patient characteristics and biochemical analyses

A BMI below 18.5 kg/m² indicates low body weight. At a 4-month treatment period, 81% of the patients achieved a BMI greater than 18.5 kg/m² and the entire group of patients reached a healthy mean value of 20 kg/m².

Significant differences were observed in the body weight, BMI, systolic blood pressure and HDL concentrations when comparing admission and 4-month follow-up measurements (Table 4.8).

Table 4.8: Biochemical variables associated with cardiovascular risk in 21 patients on admission and at follow-up (Mean and SD)

Variables	Patients on admission		Patients at follow-up		P values
	Mean	SD	Mean	SD	(95% confidence interval)
Body weight (kg)*	44.8	4.2	49.2	6.5	0.001 (6.8-2.1)
BMI (kg/m2)*	18.0	1.7	20.0	2.3	0.000 (2.6-0.8)
Systolic blood pressure (mmHg) *	98.6	11.1	104.4	12.7	0.035 (11.1-0.5)
Diastolic blood pressure (mmHg)	70.1	10.8	73.6	11.8	N/S
Total cholesterol (mg/dl)	202.0	50.0	192.3	42.5	N/S
HDL (mg/dl)*	58.8	8.7	51.7	11.2	0.033 (0.6-13.6)
LDL (mg/dl)	122.9	46.1	120.5	36.6	N/S
Triglycerides (mg/dl)	98.0	91.1	96.9	69.7	N/S
Glucose (mg/dl)	84.1	5.3	84.6	5.6	N/S
α Tocopherol (mg/dl)	1.0	0.3	0.9	0.3	N/S
Retinol (µg/dl)	45.7	14.1	41.6	17.7	N/S
Carotene (µg/dl)	6.0	4.7	6.0	4.3	N/S
Carotene (µg/dl)	18.6	17.0	18.0	14.6	N/S
Total Carotenes (µg/dl)	58.6	44.0	55.6	33.8	N/S
Lutein (µg/dl)	18.1	18.0	15.7	12.2	N/S
Crytoxanthin (µg/dl)	6.1	5.3	5.9	3.8	N/S
Lycopen (µg/dl)	9.9	5.20	9.9	5.0	N/S
Homocysteine (µmol/l)	10.3	4.4	10.5	8.1	N/S

* p: <0.05 Paired t-test Note that p< 0.01 is required to allow for multiple testing

No changes were observed in the antioxidant vitamin concentrations after four months of treatment. There were no significant differences in the hormonal profile of

patients between admission and follow-up. Oestradiol concentrations decreased during treatment, although not significantly (Table 4.9).

Table 4.9: Hormone profile in 21 patients on admission and at follows up (Mean and SD)

Hormones	Patients on admission		Patients at follow up		P values
	Mean	SD	Mean	SD	
Free T3 (pmol/l)	4.5	0.9	4.8	0.8	N/S
Free T4 (pmol/l)	12.3	2.6	11.7	2.8	N/S
Free T4/T3 ratio*	2.8	0.7	2.5	0.8	N/S
Insulin (pmol/l)	39.2	29.2	40.8	28.6	N/S
Cortisol (μg/dl)	15.4	3.3	15.0	4.8	N/S
Estradiol (pmol/l)	1.8	0.9	1.9	0.8	N/S

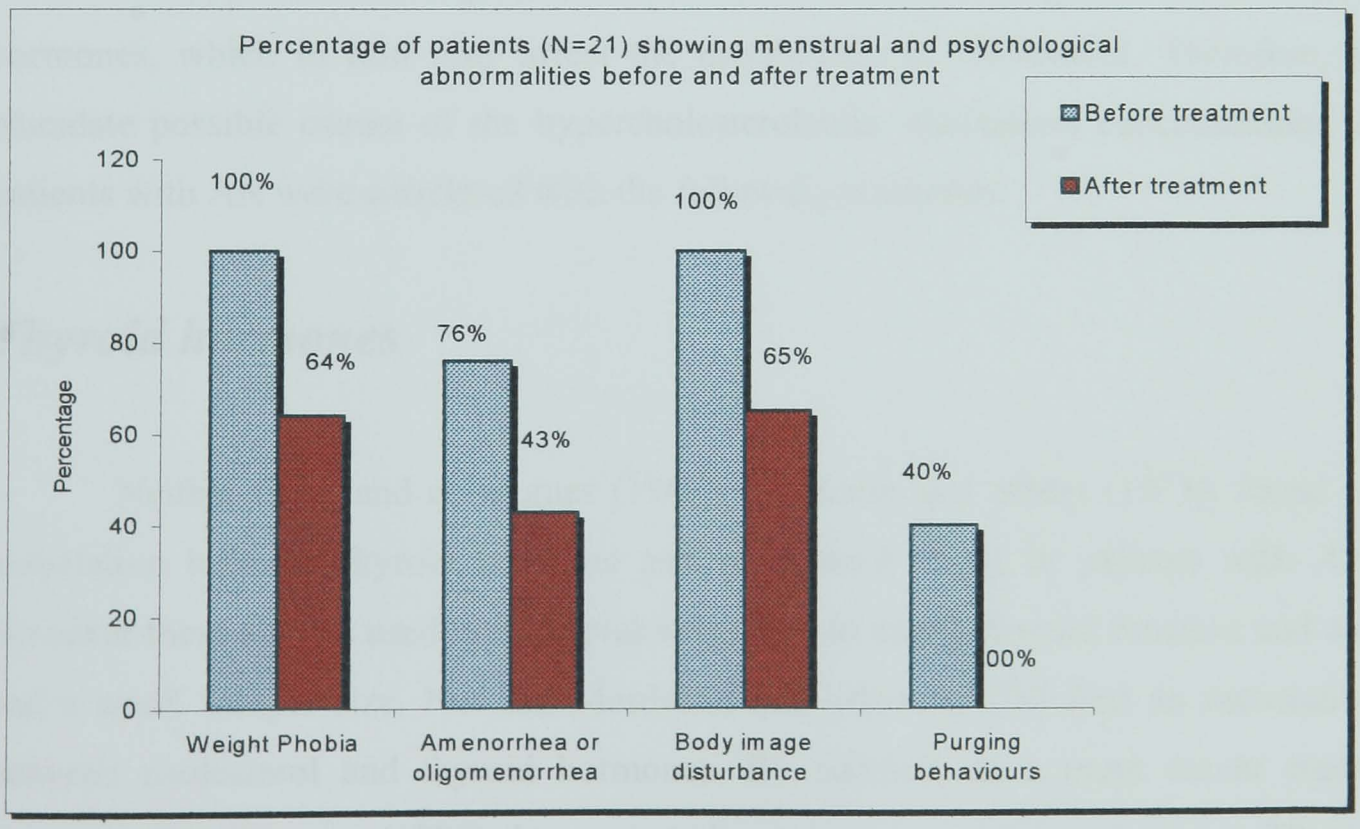
With regard to the fatty acid profile, after four months of treatment the proportions of palmitic acid decreased significantly. In contrast, DHA and total n-3 fatty acid proportions increased significantly (Table 4.10).

Psychological recovery

When the twenty-one patients were re-evaluated at the fourth month of treatment, more than half the patients still showed symptoms of weight phobia and body image disturbance. However, purgative behaviours such as vomiting, laxative, diuretics and slimming pills were eliminated in all the patients. Menstrual dysfunction persisted in 43% of patients (Graph 4.1).

Patients with menstrual abnormalities at follow up (N=9) had a lower admission BMI of 17.3 kg/m² (SD 1.0) and a higher admission TC of 239.7 mg/dl (SD 55.98) compared with patients having non-menstrual dysfunction (N=12) with a BMI of 19.0 kg/m² (SD 1.9) and TC of 173.8 mg/dl (SD 34.7). There were no associations between any other biochemical marker and the psychological indices of recovery.

Graph 4.1



4.4. Discussion

In this section the results of both studies will be discussed.

4.4.1. Lipid profile

Comparison of cholesterol values in patients and controls

According to the findings of the present study, hypercholesterolemia is to be expected in more than a third of patients with AN. TC and LDL concentrations were significantly higher in patients than in control subjects. These findings accord with the studies of Mordasini and colleagues (1978), which described type II hypercholesterolemia (high total cholesterol and LDL) in a group of patients with AN. Hypercholesterolemia II is one of the most harmful types, having the propensity to cause morbidity and mortality at a young age (National Cholesterol Education Program 2001). Although the patients had values significantly below the levels regarded as diagnostic of II hypercholesterolemia, if they continued to exhibit this cholesterol pattern the risk for cardiovascular disease would increase.

The hypothalamus regulates hormones such as thyroid, gonadal and adrenal hormones, which in turn may affect the metabolism of cholesterol. Therefore, to elucidate possible causes of the hypercholesterolemia, cholesterol concentrations in patients with AN were correlated with the following hormones:

Thyroid hormones

Neither Crisp and colleagues (1968), nor Kanis and others (1973), found an association between thyroid hormone and cholesterol levels in patients with AN. However these studies used iodine uptake methods to assess thyroid function and one had a small sample size. Nor did Mordasini and others (1978) find an association between cholesterol and thyroid hormones. By contrast, in a more recent study, Bannai and colleagues (1988) demonstrated a negative correlation between T3 and total cholesterol blood levels. This finding implies that patients with AN may suffer from the same cholesterol abnormality as patients with hypothyroidism. This present

study confirms this observation and supports the hypothesis that the low thyroid hormones contribute to the elevated cholesterol levels. Free T3 and T4 concentrations correlated negatively with total cholesterol, indicating that patients with lower thyroid levels were hypercholesterolemic. LDL levels correlated negatively with free T3 levels but not with T4, which may suggest a more powerful T3 action (which is the active form) on cholesterol metabolism.

Oestrogen

In the present study, almost 80% of patients showed altered menstrual function such as amenorrhea or oligomenorrhea on admission. Menstrual function improved at follow up, being normal in nearly half the patients. It seems that, for the full recovery of the menstrual function, more than four months of treatment is necessary. In agreement with Mira and colleagues (1988) amenorrhea at follow up was observed in patients who, on admission, had very low weight and higher total cholesterol levels. Also patients with continued amenorrhea had higher Apo A and Apo B and LDL levels were still elevated at follow-up.

Most studies have shown no association between oestrogens and cholesterol concentrations. Feillet and co-workers (2000) observed low oestradiol levels in fourteen patients with AN on admission, however oestradiol did not correlate with cholesterol levels. Their study included a small sample size, which may be insufficient to show significant associations. In the present study, concentrations of oestradiol and cholesterol correlated significantly and those patients with the lowest oestradiol values had the highest cholesterol concentrations. Homma and colleagues (2002) also explained the hypercholesterolemia in AN by a diminished secretion of estrogens and thyroid hormones which are unable to stimulate the LDL receptor to remove cholesterol from the circulation.

Decreased HDL in patients at follow up, compared with admission, may be the reason for continued amenorrhea in a number of patients. It was, therefore, hypothesised that the low levels of oestrogens at follow up might have delayed HDL catabolism, resulting in increased levels in blood. However, no association was

observed between blood concentrations of oestradiol, persisting abnormal menstrual function and HDL.

Cortisol

Similarly to the work of Feillet and co-workers (2000), this present study shows that cortisol levels were slightly elevated in patients, but not significantly, and that cortisol and cholesterol levels were unrelated. Therefore, according to these results, high concentrations of cortisol are not responsible for the high cholesterol levels in AN.

Insulin

In the present study, low insulin concentrations were found in patients with the highest cholesterol values. This finding disagrees with expectation, as high insulin levels are normally associated with elevated cholesterol values. However, this may be a consequence of malnutrition, since the most malnourished patients may have co-existing hypercholesterolemia and hypoinsulinemia.

Other theories explaining the hypercholesterolemia in AN are cholesterol retention via bile (Nestel 1974) and a reduced activity of the post-heparin lipolitic enzymes: heparin hepatic triglyceride lipase (HTGL) and lipoprotein lipase (LPL) (Klose et al 1976). Cholesterol levels may also be high in AN because the LDL receptors are inactive or are decreased in number. Fatty acid deficiency in AN produces changes in the cell membrane composition (Holman et al 1995), which may make LDL receptors located on the membrane operate abnormally.

Levels of total cholesterol and LDL were increased in restricting type patients compared with the non-restricting type. Restricting patients with AN usually have lower body weight and lower oestrogen levels than non-restricting patients and this may explain why cholesterol values were increased. Also, behaviours common to restricting types of patients such as fasting, small meal size, reduced frequency of meals, or excessive exercise, or a characteristic psychopathology may be likely to

raise the cholesterol fractions. This observation disagrees with the study of Case and colleagues (1999), which found that the abnormalities in blood lipids occurred more frequently in women who induced vomiting than in those who had not used this means of weight control.

Besides hypercholesterolemia, patients had other marginally elevated parameters associated with cardiovascular risk, such as Apo A and TG. Apo B concentrations were also elevated, probably as a result of increased LDL levels, since Apo B is the main carrier protein of LDL. The level of this apoprotein is a good measure of risk because it is the carrier of the three most atherogenic lipoproteins (LDL, IDL and VLDL) (chapter 1). TG values were not different from controls on admission and did not change significantly during treatment. This finding is dissimilar to the reports of Sanchez Muñiz and colleagues (1990) and Case and colleagues (1999), who found elevated TG in patients with AN on admission, specially in non-restricting patients, compared with controls.

Cholesterol values after treatment

The change of cholesterol values during treatment is controversial. Most of the studies showed that total cholesterol levels decreased during treatment, although not significantly (Halmi and Falk 1981; Feillet et al 2002), while others have shown the opposite (Umeki 1988; Lewis et al 1994). It is still unclear how many months of treatment are necessary to correct cholesterol concentrations to desirable values. The previous chapter demonstrated that cholesterol values decreased significantly after seven months of treatment.

The present study supports most of the previous studies, finding no significant difference in total cholesterol and LDL during treatment. There was a reduction in TC and LDL levels, which brought mean values back to desirable, but the reduction was not statistically significant.

Arden and colleagues (1990) observed that HDL concentrations increased after 16 months of treatment. Conversely, in the present study, HDL levels decreased

after four months of treatment. The difference observed between this study and the Arden and colleagues study may be due to different follow up periods and to different modalities of treatment. In this study, the decrease in HDL during treatment may be a consequence of an equal decrease in total cholesterol. It is worth noting that HDL values decreased but not significantly after adjusting for multiple testing. Increased HDL levels are expected when there is an increase in oestrogens, which were slightly increased at the follow up compared with admission levels; an increase in polyunsaturated fat intake (Vega et al 1982) or exercise (Kaplan et al 1985). It could, therefore, have happened that HDL levels were high on admission because the patients over-exercised and these high levels returned to normal during treatment.

In conclusion, the causes of elevated total cholesterol and LDL levels in patients with AN remain uncertain. This thesis helps in clarifying the evidence on the effects the hypothalamus on cholesterol metabolism. Low blood concentrations of thyroid hormones, insulin and oestradiol were proved to be associated with high cholesterol levels. The unchanged concentrations of these hormones after re-testing patients may explain the consistent cholesterol levels between admission and follow-up. There are many areas that require further investigation, such as studies on cholesterol balance, LDL receptor and cholesterol related enzymes and hormones.

Some authors (Mehler et al 1998 and Rock and Currran-Celentano 1994) supported the premise that the cardiac abnormalities associated with AN are totally unrelated to the cardiac problems associated with hypercholesterolemia. Cardiac complications in AN, such as arrhythmia and altered blood pressure, are not linked to hypercholesterolemia. However, this study has demonstrated that many risk factors for cardiovascular disease are elevated in patients with AN and hypercholesterolemia is one of these. Hypercholesterolemia may not be considered a serious problem to address during treatment because it does not show immediate consequences. However, after many years of suffering from AN and from elevated blood lipids, the likelihood of cardiac problems increases.

Klag and co-workers (1993) observed that high cholesterol levels at the age of 22 years predict cardiovascular disease in middle age and this suggest that risk factor

modification should be initiated at early ages. Unfortunately at this age young people are unlikely to be concerned about risk of cardiovascular disease, which to them seems remote. In the particular case of patients with AN, they are not afraid of the risk of under-nutrition and they are unlikely to be concerned about promoting health and preventing heart disease.

4.4.2. Fibrinogen

Fibrinogen concentrations were found to be higher in patients with AN than controls, but differences were not significant when values were adjusted for age and BMI (kg/m^2). One reason for fibrinogen being slightly elevated may be that, as it is an acute phase protein, the synthesis could be enhanced in these patients as a result of the stress of malnutrition. If AN is not treated and elevated concentrations of fibrinogen continue, this may be a factor contributing to increased thrombogenesis and obstructing the blood flow to the heart.

In the Framingham Study, a 12-year follow-up showed that the risk of developing cardiovascular disease was associated with an antecedent fibrinogen value higher than 696 mg/dl. The mean fibrinogen value at baseline for women with no cardiovascular disease was 297 mg/dl and 322 mg/dl for women with prevalent cardiovascular disease (Kannel et al 1996). In this study the mean fibrinogen value for patients was 350 mg/dl.

Anyan (1974) found low levels of plasma fibrinogen in seven girls with AN; during weight recovery these levels returned to normal. The authors suggested that, in AN, there is a decrease in production of fibrinogen. Similarly, Ogston and co-workers (1976) found low levels of fibrinogen in a sample of patients with AN (N=7) compared with healthy individuals. However, these studies had small sample sizes and did not assess other risk factors for cardiovascular disease. Fibrinogen values may be marginal in AN because, as previous research has shown, it tends to cluster the major atherogenic risk factors such as hypercholesterolemia and tobacco smoking. However, no correlation was found in this study between fibrinogen and cholesterol levels or tobacco use. High blood fibrinogen levels are generally determined

genetically (Hamsten et al 1987) or by an impaired degradation in the circulation (Verschuur et al 2000). It is therefore likely that patients with AN have difficulty with fibrinogen clearance, or that this is a particular group of patients with genetically elevated blood fibrinogen.

4.4.3. Homocysteine

Effective metabolism of homocysteine requires an adequate supply of vitamin B6, B12 and folate. It was, therefore, expected that there would be high blood concentrations of homocysteine in patients due to insufficient intake of these vitamins. However, plasma homocysteine concentrations were not found to be significantly higher in patients compared with controls. Homocysteine may not be elevated because of the adequate diet, including meat, fresh vegetables and cereals, that the patients began to eat during the first month of treatment. This diet may have already restored vitamin levels and consequently normalised homocysteine values. Naurath and colleagues (1995) found a reduction in homocysteine levels in elderly subjects after 3 weeks of folate, B12 and B6 supplementation. Schorah and co-workers (1989) observed a 10% reduction in homocysteine in healthy subjects consuming fortified cereals for four weeks. Therefore, homocysteine may be only elevated at baseline in untreated patients with AN at the peak moment of their illness when they exercise restraint in eating.

The age of the patients may also have exerted a protective effect on homocysteine. Homocysteine values are mostly elevated in old age, and younger patients have been shown to have lower homocysteine concentrations.

A slow progression of vascular disease may be predicted for those cases exceeding the desirable values. Nygard and colleagues (1997) demonstrated that the likelihood of mortality was strong in patients suffering from vascular disease with homocysteine levels greater than 15 μmol/l. A meta-analysis of the observational studies of homocysteine concentrations and vascular disease suggested that a lowering of plasma homocysteine concentrations by 1 μmol/l was associated with a reduction of 10% risk for vascular disease throughout the range 10-15 μmol/l (Boushey et al

1995). The value of the 50th centile for the normal distribution of homocysteine in 135 healthy women aged below 45 years was 8.1 μmol/l (Refsum and Nygard, 1998). The mean concentration of homocysteine in patients was 10 μmol/l, which is within desirable levels, and this value did not change during treatment.

Few studies have been carried out on homocysteine in AN. Moyano and co-workers (1998) measured homocysteine, vitamin B12 and folate levels in a group of 43 adolescent females with AN. They found high levels of plasma homocysteine, compared with reference values, but did not find significant vitamin deficiencies. The mean homocysteine value was 10.3 μmol/l, which is similar to the mean value of the patients in this study. The authors proposed, for these patients, a sub-clinical folate deficiency, caused by intracellular folate deficiency. Homocysteine levels were completely corrected after nutritional rehabilitation. Conversely, Matzkin and colleagues (2001) observed no significant differences in homocysteine levels in a small sample of patients with AN (N=8) compared with controls. However, levels of homocysteine exceeding 16 μmol/l were present in nearly 60% of the patients compared with 30% of controls.

4.4.4. Lipid-soluble antioxidant vitamins

Fat-soluble vitamins E and carotene exert antioxidant activity through protecting LDL from oxidation; breaking the free radical chain reactions. Patients with AN are at greater risk of oxidative damage due to an insufficient intake of antioxidant vitamins coupled with psychological stress and excessive exercise induced stress (Moyano et al 1999).

Tocopherol

The hypothesis was proved that tocopherol concentrations were lower in patients compared with controls after adjusting for cholesterol values. Similarly, Langan and Farrell (1985) reported low levels of and γ tocopherol in a group of patients with acute AN. However, levels of α tocopherol, which is the most active form of Vitamin E, did not differ from the control subjects. Moyano and co-workers (1997) found low

levels of α-tocopherol and concluded that this may be due to low vitamin intake and oxidative stress during a prolonged period. Similarly, Rock and Vasantharajan (1995) found low plasma α tocopherol levels in patients with AN. The authors did not find any changes when baseline and discharge (2-6 weeks after admission) vitamin values were compared. Vaisman and colleagues (1992) observed that the low tocopherol levels in patients with AN increased during re-feeding. In the present study there were no observed changes in the vitamin concentrations after four months of treatment, which suggests that a longer period is needed for the vitamin levels to correct to desirable values.

Carotene

Hypercarotenemia is a common finding in AN. The cause of hypercarotenemia is still unknown. Patients usually exhibit a yellow hyperpigmentation in the skin considered to be harmless, called xeroderma (Crisp and Stonehill 1967). Hypercarotenemia has been considered to be related to under-nutrition; however, two studies proved this assumption to be wrong. Key (1950) did not find any changes in retinol and carotene levels in starved volunteers and concluded that the need for these vitamins may be reduced during starvation. Robboy and colleagues (1974) demonstrated that patients with cachexia had low carotene levels, while patients with AN had hypercarotenemia.

A number of studies have investigated the causes of hypercarotenemia in AN. Some of the possible hypotheses, explaining the elevation of carotene, are discussed below:

1. It has been suggested that patients ingest large quantities of low calorie food, such as carrots and green vegetables, which may lead to high carotene levels (Dally 1954 and Crisp and Stonehill 1967). However this assumption was discarded by many authors (Rock and Swendsied 1993; Curran-Celentano et al 1985; Thibault and Roberge 1987), who measured food intake and blood carotene levels and did not find that hypercarotenemic patients were eating higher amounts of carotene-rich food. High intake of carotene foods may be the cause of hypercarotenemia in some but not

all patients with AN. The present study demonstrates a higher concentration of total carotenoids, lutein and cryptoxanthin, which are bio-markers for the intake of yellow-orange fruits and vegetables (peaches, carrots, pumpkins, melons, oranges) and dark green vegetables (spinach).

2. Bhanji and Mattingly (1981) observed lower concentrations of carotene in patients who vomited compared with the dieting type of patients. Rock and co-authors (1996) found that women who dieted, avoiding the consumption of dietary fat, had the highest carotene concentrations. Therefore, hypercarotenemia may be a consequence of an eating behaviour characterised by dieting or low fat intake.

3. Rock and Swendsied (1993) tested the hypothesis that hypercarotenemia was caused by an enhanced carotene absorption. They gave carotene supplements to patients with AN and controls and measured the plasma vitamin levels, finding no differences between groups.

4. Curran –Celentano and co-workers (1985) suggested that the hypercarotenemia in AN was caused by low T3 concentrations. This is supported by the fact that in many diseases where thyroid activity is impaired, such as hypothyroidism, diabetes, nephrotic syndrome and hypopituitarism, patients present altered carotene levels and xeroderma. There is also some evidence that carotene conversion to retinol is influenced by thyroid hormone and that thyroxine affects the storage of retinol and the rate at which is used. Carotene and thyroxine have been proved to have opposing actions on the growth of rats deficient in vitamin A (Euler and Klussman 1932). A negative correlation was observed between β-carotene and free T3 and this was also significant when carotene was expressed as carotene/total cholesterol ratio ($p= 0.019$, $r= -0.381$ Pearson correlation). Elevated levels of carotene may therefore be explained by a decreased thyroid hormone activity.

5. Another theory is that there is a decreased tissue storage capacity (Rock and Swendseid 1993). Retinol and carotene levels were higher in the patients with the lowest BMI, suggesting that, in the thinner patients, there is an increased release of carotene from the fat deposits to the blood because of reduced adipose tissue.

6. Elevation of carotene may be explained by a decreased catabolism of LDL, which is the main carrier of carotene in the blood. The release of lipids and carotene from fat deposits, or the failure to metabolise lipoproteins in the cells, may increase the levels of carotene in the blood. However, Banghi and Mattingly (1981) and

Boland and colleagues (2001) observed that the elevated carotene levels did not appear to be associated with the serum levels of cholesterol. In the present study carotene levels were not significantly higher in patients than in controls after adjusting for total cholesterol values. This suggests that hypercarotenemia is a reflection of increased blood lipids and that cholesterol is a confounding variable when measuring carotene values in AN.

Retinol

Studies on retinol concentrations in AN are also controversial. There are papers reporting normal (Rock and Vasantharajan 1995, Casper et al 1980, Langan and Farrell 1985 and Philipp et al 1988), lower (Silverman 1974; Abraham et al 1980 and Vaisman et al 1992) and higher (Binsbergen et al 1988 and Mira et al 1989) retinol concentrations in patients compared with controls.

In the present study, as was predicted, plasma retinol concentrations in patients were significantly lower than in control subjects. This finding agrees with Silverman (1974) who observed low levels of retinol in patients with AN. Vaisman and colleagues (1992) also found similar results and noted that retinol levels increased during re-feeding. In the present study, conversely, there was no significant improvement over time. Patients might have adapted to long-standing inadequate intake of retinol, depleting retinol storage in the liver and maintaining low blood levels. There may be a need for a lengthy treatment period to reverse retinol status.

The low levels of retinol in AN may be an acute-phase response to malnutrition and stress, since decreased retinol is also seen in subjects after myocardial infarction, infection and trauma (Ruiz Rejón et al 2002). Low retinol levels may be a result of the reduced hepatic synthesis of retinol carrier protein. Abnormal retinol-binding protein has been postulated to alter retinol concentrations. Levels of this protein depend highly on pre-albumin (transthyretin) concentrations, which are usually low in AN (Feillet et al 2000). However, this theory has not been proved. Another possible explanation for the decreased retinol concentrations is that there is a low conversion rate from carotene to retinol. Hypercarotenemia may occur

because of an error of the metabolism in the enzyme 15, 15'-dioxygenase to convert carotene to retinol. Another theory may be that retinol is taken by the immune system to combat infection, since decreased immune status has been documented in AN (Marcos et al 1997). A different body distribution of retinol, such as an increased distribution in the extra-vascular space, may also explain the decreased blood retinol values.

In summary, according to these results, vitamin antioxidant levels in AN were characterised by low α tocopherol and retinol and by high but not significant carotene concentrations after adjusting for TC. Vitamin status did not change after four months of treatment, which may be a consequence of a long lasting energy and nutrient deficiency. A longer rehabilitation period may be required to normalise these values.

4.4.5. Fatty acids

At the present time there are two published studies on fatty acids in AN (Lagan et al 1985 and Holman et al 1995). These investigations include a small number of patients (N= 17, and 8 respectively) and do not specify the length of time that patients have been undertaking treatment before the measurement. The main findings of Lagan and co-workers were low EFA and *n*-6 derivative levels. Holman and colleagues observed low proportions of both *n*-3 and *n*-6 derivatives and high concentrations of mead acid. Although EFA deficiency was not observed in the present study, other fatty acid abnormalities were evident. This study did not support the finding of Holman and colleagues showing normal levels of mead acid and high *n*-3 fatty acids in patients with AN compared with controls.

Two theses from King's College London (Farrant 2000 and Neville 2002) reported lower DHA levels in both plasma and erythrocyte membranes in patients with AN compared with controls. In the present study, DHA proportions were not significantly different in patients compared with controls. Total *n*-3 fatty acids were increased and the EPA proportions were significantly higher. However, in controls, EPA proportions were below the reported values for USA females (Holman et al

1979). Comparison with these reference values is limited because they are based on a clinical population from a different country and dietary habits are likely to have shifted in the last 20 years. The Argentinean diet is characterised by low fish intake and high intake of meat (beef) and this may explain the higher, although not significantly, saturated fat in controls eating a varied diet.

Total *n*-3 fatty acids, EPA and DHA proportions increased in patients after four months of treatment. This is probably the effect of a varied diet provided to patients during treatment, which included fish oils.

Wilson and colleagues (1989) reported that a 4-week weight loss in obese individuals was followed by increased serum phospholipid arachidonic acid levels, which suggests that, during weight loss, there is a higher mobilisation of arachidonic acid from the tissues. This may in part explain the present findings of reduced arachidonic acid proportions in patients with AN on admission, when compared with follow up. Weight loss for a substantial period of time, such as is the case with these patients, may have caused arachidonic acid to mobilise and catabolise, which was then reflected in a low plasma concentration on admission. A reduced activity of the enzyme $\Delta 5$ desaturase and decreased arachidonic acid levels have been shown to occur in fasting rats (Pugh and Kates 1984). Arachidonic acid proportions increased during treatment, again this is probably explained by the consumption of a varied diet. Since *n*-6 fatty acids decrease cholesterol concentrations (Kris-Etherton and Yu 1997), arachidonic acid deficiency may be involved in the hypercholesterolemia of AN. The abnormalities in the fatty acid profile in AN are probably a factor in the elevation of cholesterol and may, therefore, be another contributor to cardiovascular disease in the disorder.

There are a number of explanations for the differences observed in the fatty acid profile of patients compared with controls. Nutritional, hormonal and other factors such as medication, smoking and alcohol have all been shown to affect fatty acid concentrations. Hormones are involved in the modulation of the elongation and desaturation reactions (Brenner 1981) and the abnormal hormone profile in AN may lead to the effect on the fatty acid profile.

According to the results, treatment should be accompanied by a diet rich in *n*-6 fatty acids and an adequate food intake to normalise the fatty acid profile and improve cholesterol levels. Vegetable oils (sunflower, safflower, corn and soyabean oils) provide linoleic acid, and egg yolk, meat and fish are all sources of arachidonic acid, which patients were lacking. Arachidonic acid deficiency in patients on admission possibly reflects a low consumption of food items containing this fatty acid, while the high *n*-3 fatty acids may be a result of an increased intake of green leaves and fish. Subjects with AN usually consume a diet rich in vegetables and fruits and avoid oil and fat (Moreiras Varela et al 1989) and red meat intake (O' Connor et al 1987; Hadigan et al 2000). These are all dietary choices that may alter the plasma fatty acid profile. Data from this thesis suggests that a balanced diet, coupled with psychiatric treatment during a sustained period, is probably sufficient to correct these abnormalities without the need for supplementation.

4.4.6. Psychological variables at follow up

At a 4-month treatment period most of the patients had recovered their weight. However, only 21 out of 30 patients were followed up due to drops out, and this sub-group was less ill, having a higher mean admission weight. Many symptoms, such as disturbances in body image and weight phobia, persisted after the follow up. Purging behaviours (vomiting, laxative, diuretics and slimming pills) had been eradicated. 43% of the patients were still experiencing menstrual dysfunction at follow up and this was more frequent in patients with the lowest BMI and highest cholesterol levels on admission. Frisch (1990) observed that young adult women need a fat content of 22 to 25% for menstruation to occur. Golden and colleagues (1997) described that menstruation in patients with AN re-establishes itself at a mean of 9.4 months after treatment, when patients gained 2.05 kg more than the weight at which menses ceased. It would, therefore, take more than four months to recover psychological health and to re-programme the biochemical marker into desirable ranges. Since length of treatment for AN ranges from 2-3 years (Russell 1973), it would be interesting to measure atherogenic markers at regular intervals during treatment to predict at which point of treatment these normalise.

4.5. Limitations and recommendations

Some of the hypothesised relationships (for example hypercholesterolemia and high cortisol levels) or the expected changes during treatment might not be demonstrated given the small size of the sample. Another limitation is that many patients included did not fulfil the criteria for AN completely at the time of the investigation. Therefore, some of the variables tested were not that abnormal on admission and did not change as much as expected at follow up.

Some patients were taking psychiatric drugs during treatment, and four control subjects were taking contraceptive drugs, which could have affected the blood results. However, no significant differences in any of the tested parameters were observed between subjects who took contraceptive pills and those who did not. Also, patients were recruited during the month of admission and they might have already changed their altered anorexic unhealthy eating and behavioural patterns, such as food restriction and intensive exercise. This might have been the major factor confounding the results. Unfortunately, the dietary composition of patients could not be assessed because Aluba disapproved of requesting patients to quantify food intake.

Oestradiol levels were assessed at different stages of the menstrual cycle and this may be a confounding variable, especially in control subjects who had normal menstrual cycles. Oestradiol levels are known to increase during the follicular phase of the cycle. However, as subjects were assessed randomly, an average value of oestradiol in different phases of the menstrual cycle was expected. Furthermore, the main goal in the determination of oestradiol was to obtain a mean concentration in patients to correlate with cholesterol values.

Plasma fatty acid determination informs about the circulating levels of fats that are being transported from or to the adipose tissue. The measurement of fatty acid in the red cells is a preferable means of assessing fatty acid metabolism, because it gives longer retrospective information, but this could not be done in the present study for cost considerations.

Limited information is available on biochemical variables in recovered patients. There is a need to estimate morbidity and mortality associated with cardiovascular disease in patients who had been ill for a long time. The grade of recovery and the risk and symptoms of cardiovascular disease should be assessed in a follow up study for up to 20 years. AN is an illness that became prolific only 40 years ago (Moller-Madsen and Nystrup 1992) and, therefore, retrospective studies are lacking. The present study has followed up patients for a short period of four months, and changes should be expected further on in the treatment. Some of the difficulties of the follow-up were that patients dropped out from treatment, refused to participate and the research fieldwork was scheduled for only one year. An interesting study would be to follow up the patients of the present study in 30-40 years time to investigate the long-term effect of AN on cardiovascular disease, performing, for example, another lipid profile and hormonal testing and recording morbidity.

Patients who refuse to undertake treatment and remain at a low weight could pass on the risk of cardiovascular disease to their descendants. Women with AN have fertility problems, although some of them may menstruate and conceive while maintaining a low weight. Low average weight gain during pregnancy (around 8 kg), impaired intrauterine growth and small babies at birth have been observed in patients with AN (Steward et al 1987 and Treasure and Russell 1988). Foetal under-nutrition leads to impaired foetal growth and contributes to cardiovascular disease in later life. Low birth size babies cannot adequately develop the myocardial muscle. Consistent relationships have been found between low birth size and several factors that are markers for cardiovascular disease. These markers include hypertension (Barker et al 1990), impaired glucose tolerance testing (Hales et al 1991), elevated lipid concentrations (Barker 1993) and disordered blood coagulation (Barker 1995). Cardiovascular disease may, therefore, be transmitted from malnourished mothers with AN to their children. This is a new field that requires further research.

Further studies looking at cholesterol absorption, metabolism and excretion in patients with AN are needed. Studies investigating the dietary intake and exercise levels (using techniques such as accelerometry) before patients undertake treatment are also necessary.

4.6. Conclusion

This study has shown that some biochemical parameters associated with cardiovascular disease, such as total cholesterol, LDL and Apo B, were elevated in a group of patients with AN on admission, while antioxidant vitamins (retinol and tocopherol) were decreased. This suggests that these patients are more vulnerable to cardiovascular disease morbidity and mortality. At the onset of AN, the risk factors for future diseases might not be taken into account by the patient, family and professionals. Cardiovascular disease is not usually seen in these patients when studied because they tend to be young and may not, therefore, have entered the period of high risk for progressive cardiovascular disease. Nevertheless, some studies have shown that cardiovascular disease occurs in patients with AN in their thirties (Moesli 1967 and Isner et al 1987). In addition, there is a lack of retrospective studies investigating the consequences of risk factor exposure in subjects with AN. Eating disorders may become a modifiable risk factor and preventable cause of future cardiovascular disease (Sullivan, et al 1998).

Cholesterol values were shown to be related to low thyroid and gonadal hormonal levels, indicating slow metabolism due to fasting. The majority of patients (80%) decreased cholesterol levels from admission to follow up. Only four patients had higher cholesterol levels at follow up compared with admission and two of these had very low cholesterol levels on admission (<135 mg/dl). It could therefore be suggested that cholesterol tends to correct to desirable levels during treatment. After four months of treatment, the low body weight had improved and there was a tendency for most of the elevated biochemical parameters to have decreased. However, more than a 4-month follow up is needed to observe any other biochemical or psychological changes.

Elevated total cholesterol levels were related to the age of the patients, and the time patients had been ill. Treatment has been shown to lower cholesterol levels and diminish the risk of cardiovascular disease, at least in young patients. As patients get older, and without adequate treatment, this risk may increase as they enter the age

range of higher probability for suffering from cardiovascular disease. Patients may be at risk if they remain at a low weight.

Health professionals need to be more aware of the long-term risk factors of AN, especially in adolescents. The elevated cardiovascular markers may not have short-term consequences for the patient. However, if AN is not treated in its early stages, and the illness persists when the patients are older, the risk factors would be intensified. It may be prudent to inform subjects suffering from AN that they could be at risk from the complications of long-standing elevations of a cluster of risk factors for cardiovascular disease. High cholesterol levels, for example, may alert patients to the gravity of the illness, especially in chronic cases; thereby encouraging them to undertake treatment.

In summary, patients with AN exhibit a number of risk factors for cardiovascular disease. Psychiatric treatment and an adequate dietary plan over a lengthy time will probably correct the biochemical abnormalities and reduce the atherogenic risk.

Chapter 5 - Treatment assessment

(Study 5)

Chapter 5 - Treatment assessment

5.1. Introduction

Patients with AN have biochemical (high cholesterol, hyperhomocystenemia, low blood essential fatty acid levels, decreased blood antioxidants levels) and behavioural (tobacco smoking, high coffee consumption, low EI) risk factors for cardiovascular disease. In the dietary treatment for AN, dietary fat as a risk factor for cardiovascular disease is generally not considered. Patients with AN are usually fed large quantities of high-energy density food, rich in energy and saturated fat in order to achieve a weight target rapidly (Russell 1998; Touyz and Beaumont 1985). These types of diets also predispose to overweight and obesity that, in young people, are associated with future cardiovascular disease (Hubert et al 1983; Manson et al 1995). Neither are there treatment norms for the use of tobacco, alcohol, coffee and other risk behavioural patterns. Vegetarianism, a common choice among eating disorder patients (O´Connor 1987), is generally accepted during treatment in spite of the harmful effects of some vegetarian diets. Vegetarian subjects tend to have a low energy intake and body weight (Hebbelink et al 1999) compared with omnivores and are at greater risk of nutritional deficiencies, cardiovascular disease (Hung et al 2002) and osteoporosis (Barr et al 1998).

In AN, there are also risk factors for osteoporosis (Chapter 1). Patients with AN have both decreased bone formation and increased bone resorption (Powers 1999). The loss of bone mass is a major and evident problem in the management of these patients (Grinspoon et al 1997), predisposing to an inadequate peak bone mass in adolescence and early adulthood, and to bone fracture.

Despite the presence of risk factors for cardiovascular disease, no review study has been undertaken on whether treatment programmes to address cardiovascular problems might be appropriate. Patients with AN do not often die of cardiovascular disease and this is not an immediate complication, therefore no preventive measurements during treatment have been proposed. There have been some

guidelines for the treatment of osteoporosis because it affects about half patients with AN (Grinspoon et al 1997) and is an immediate complication.

This chapter aims to review the extent to which two chronic diseases: osteoporosis and cardiovascular disease are considered in Argentinean treatments for AN. The objectives of this study were:

- To identify what the eating disorders treatment institutions estimate to be the short-term and long-term complications of AN
- To identify the key features of the dietary management of patients with AN
- To examine whether treatments for AN include advice concerning risk factors for cardiovascular disease and osteoporosis

5.1.1. Hypotheses

Primary hypothesis

- Treatments for AN do not address the following potential risk factors for cardiovascular disease and osteoporosis: tobacco smoking, coffee and alcohol drinking, salt intake, vegetarianism, overweight after re-feeding, low calcium intake, and the high saturated fat intake.

Secondary hypotheses

- Cardiovascular disease and osteoporosis are not recognised by the institutions as serious problems in AN

- Cardiovascular disease is not perceived by the institutions as a long-term risk of AN

- The provision of a balanced diet with adequate amounts of carbohydrates, protein and fat is one of the least important priorities of treatment

- A low saturated fat dietary plan is not prescribed to patients with hypercholesterolemia

- A high fat dietary plan is prescribed to patients to rapidly recover weight loss

- According to the estimates by professionals in charge of the nutritional treatments, more than 25% patients become overweight (BMI >25 kg/m^2) after re-feeding

- The calculation of the patient's energy and macronutrient requirement only takes place in those institutions working with dietitians

- A dietary plan rich in Calcium is not prescribed to patients with bone loss

5.1.2. Design and Sample

Twenty-five well-known institutions treating AN in Buenos Aires (Argentina), identified via the Internet or by psychiatric reference, were contacted. Only thirteen institutions agreed to participate and completed a questionnaire. The questionnaire, designed to achieve the objectives of the study, consisted of open and closed-ended questions and was divided into two parts: 1) a general section, and 2) a nutritional section (Appendix 5. 1).

1) The general section aimed to:

- Identify the institutions treating AN; how many patients they currently treat, and the type and length of treatment offered.
- Investigate the diagnostic criteria for AN used.
- Estimate the frequency of cardiovascular disease in AN; determine whether professionals think that this is a serious problem during treatment; and investigate the management of hypercholesterolemia.

- Estimate the frequency of osteoporosis in AN; determine whether professionals think that this is a serious problem during treatment; and investigate the management of bone loss.
- Compare awareness of the professionals in the short and long term risk of AN with the prioritisation they give to different forms of treatment.

2) The nutritional section aimed to:

- Identify the professionals giving dietary advice.
- Identify the advice they give.
- Ascertain if and how energy intake, macronutrients and portion size are estimated.
- Investigate whether they set a target weight, if this was achieved and how obesity due to re-feeding is managed.
- Ascertain if they take into consideration the practice of risk behavioural patterns for cardiovascular diseases and osteoporosis: tobacco smoking, coffee and alcohol drinking, salt intake, vegetarianism, overweight after re-feeding, low calcium intake, and high consumption of saturated fat.

5.1.3. Methodology

The reasons of choosing a questionnaire to assess the type of treatment offered were that it would : 1) reach all the members of the eating disorders team, so it could be discussed in a team meeting and 2) simplify completion, so it could be done whenever the professionals had spare time. The papers of Robinson (1993), Herzog (1992) and the Eating Association Guide (Hogg 1995) were used as models to design the questionnaire.

Two psychiatrists working in eating disorders units in London were consulted regarding the implementation of the questionnaire. They advised approaching the co-ordinators of the eating disorder teams in Buenos Aires asking them to discuss the questionnaire in a meeting with their teams.

Therefore, the co-ordinators of the eating disorder institutions were contacted by telephone. The co-ordinators usually had the role of providing admission and discharge planning for patients and supervising all aspects of the patients' care. It was explained that this study aimed, through a questionnaire, to review the extent to which osteoporosis and cardiovascular disease were considered in the treatment of patients with AN. When the co-ordinators agreed to participate they were sent the questionnaire by post. The coordinators introduced the questionnaire in a professional meeting to all the other members of the eating disorders team and passed it to the clinician and the dietician. The clinicians filled in the general part of the questionnaire, and the dieticians or doctors specialising in nutrition completed the nutrition section. The head of the eating disorders team signed a consent form agreeing to participate in the study.

The co-ordinators were responsible for collecting the questionnaire and to make certain that all the questions were answered. A personal interview was arranged with the co-ordinators to obtain the completed questionnaire and to discuss any conflicting points.

This study was scheduled to run from July to September 2001 and ethical approval was obtained from King's College London (99/00-91), where the study was designed.

5.1.4. Statistics

Descriptive statistics were used to describe the characteristics of the offered treatments. Pearson's correlation was used to associate two independent variables (for example: presence of dietitian in the institution and calculation of energy requirements).

Declaration

The author of this thesis designed the present study, carried out the fieldwork and analysed the data.

5.2. Results

5.2.1. Characteristics of the institutions

The sample comprised of thirteen institutions treating AN in the city of Buenos Aires (Table 5.1).

Table 5.1: Characteristics of the institutions

Name of institution	N patients currently treated	N AN admissions last month	Mean duration of treatment in and out-patients (years)	Type of institution
Aluba	120	30	4	Private/State
Argerich Hospital *(Adolescence Service)*	10	1	2	State
Pirovano Hospital *(Food Service)*	19	10	1.5	State
Frances Hospital *(Psychiatric Department)*	12	5	4	State
Durand Hospital *(Adolescence Service)*	10	5	2	State
Gutierrez Hospital *(Adolescence Service)*	70	6	2	State
Nutritional Support Centre	4	0	No time limit	Private
Borda Hospital *(Eating Disorders Unit)*	15	0	2	State
Italiano Hospital *(Nutrition Department)*	35	12	2	State
FUNTAPID	15	2	2.5	Private
Clinicas Hospital *(Nutrition Department)*	15	3	2	State
University of Buenos Aires *(Health Division)*	2	3	1	State
Oro Centre	10	2	3.5	Private

All the institutions treated AN, BN and Eating Disorders Non-Otherwise Specified. Ten institutions also offered treatment for obesity and eight institutions treated Binge Eating Disorder. The DSMIV was used by all the institutions (except one) to diagnose AN. 38% (N= 5) of institutions had hospitalisation facilities, another 38% (N= 5) offered day-patient treatment and all the institutions provided out-patient treatment.

5.2.2. Estimation of short and long-term complications of AN

To the question: which do you think are the three most important short-term risks of AN, the majority of the institutions answered that these were electrolyte imbalance, arrhythmia and nutritional deficiencies. The three most important long-term risks of AN were osteoporosis, infertility and nutritional deficiencies. Cardiovascular disease was not perceived by the institutions as a long-term risk resulting from AN, as had been expected, though osteoporosis, in contrast, was perceived as a long-term risk by all the institutions (Table 5. 2).

Table 5.2: Respondents' estimation of the three most important short and long-term health risks in AN

Medical complication	Short-term risk		Long-term risk	
	N	%	N	%
Electrolyte imbalance	10	77	2	15
Arrhythmia	9	69	3	23
Nutritional deficiency	8	61	5	38
Osteoporosis	2	15	13	100
Depression	2	15	1	8
Hypoglycemia	3	23		
Low immunity	1	8		
Gastrointestinal complications	1	8	2	15
Sudden death	1	8	1	8
Hormonal complications			3	23
Infertility			5	38
Stunted growth			1	8
Grave psychopathologic picture			3	

When the institutions were asked which were the three most important priorities of treatment, three institutions did not answer the question, arguing that all the optional statements were priority number one in treatment. Of the institutions which answered this question, 90% (N=9) reported that among the three most important priorities of treatment was to normalise clinical parameters, 70% (N=7) to eliminate abnormal eating behaviour, 30% (N=3) to eliminate substance use, 30% (N=3) to restore weight loss, 30% (N=3) to encourage the intake of frequent meals, 20% (N=2) to encourage personal relationships, 20% (N=2) to treat depression and 10% (N=1) to promote a balanced diet. To induce menstruation was not selected as a priority of treatment by any institution. It was expected that the provision of a balanced diet with adequate amounts of carbohydrates, protein and fat would be one of the least important priorities of treatment. This assumption was proved to be true, because only one institution selected this point as one of the three most important priorities in treatment.

Table 5.3: Respondents' estimation of how often the institutions see medical problems in patients with AN

Medical problem	Estimation of how frequent the problem Percent			
	Less than 25%	25 to 50%	51 to 75%	More than 75%
Blood cholesterol above 200 mg/dl	38	31	15	15
Arrhythmia	85	15		
Hypotension	8	31	23	38
Chest pain	85	15		
Bone fracture	100			
Bone loss	46	31	23	

A third of the institutions reported seeing hypercholesterolemia in more than half the patients (Table 5.3). All the institutions tested for cholesterol levels on admission and during treatment over a mean period of 4 months, SD: 1.8. Hypercholesterolemia was treated with medication by 23% (N=3) of institutions, with dietary plans by 77% (N=10) and with exercise by 69% (N=7). The hypothesis that a

low saturated fat dietary plan was not prescribed to patients with hypercholesterolemia could not be proved, because more than half the institutions declared that they were treating high levels of cholesterol with an adequate dietary plan. When the patient was hypercholesterolemic, 54% (N=7) of institutions investigated cholesterol levels in the patient's family by asking the parents to have blood tests.

Ninety two percent (N=12) of institutions reported that cardiovascular abnormalities were a serious problem in AN and 100% that bone abnormalities were a problem. The hypothesis that cardiovascular complications were not seen by the institutions as a serious problem in AN could not, therefore, be proved. Bone loss was appreciated as being considerably prevalent (Table 5.3). 85% (N=11) institutions treated bone abnormalities with medication (for example: calcium supplements and oestrogens), 92% (N=12) with a dietary plan, 69% (N=9) with exercise and 8% (N=1) with sun bathing. Therefore, the assumption that a dietary plan rich in calcium is not prescribed to patients with bone loss is not accepted since more than 2/3 of the institutions claimed they were prescribing an adequate diet rich in calcium to patients with bone loss.

5.2.3. Nutritional information

In this section the results of the dietary treatment for patients with AN, and the advice provided in relation to the cardiac and osteoporotic risk will be shown.

5.2.3.1. Weight management

Forty six percent (N=6) of institutions set a desirable weight for the patients on admission, while 85% (N=11) set a desirable weight during treatment. The institutions reported that a mean of 63% (SD: 19 N=9) of patients reached a target weight, 58% (SD: 18 N=8) of patients maintained this target weight, 40% (SD: 28 N=8) had remissions and only 7% (SD: 9 N= 13) of patients became overweight after re-feeding. The hypothesis that more than 25% of patients became overweight (BMI >25 kg/m^2) after re-feeding is, therefore, rejected. However, this information is

contradictory because when they were asked in the questionnaire whether being overweight was a significant problem in patients after re-feeding, 53% (N=7) institutions answered positively. The institutions possibly considered 7% of patients becoming overweight a significant percentage. With regard to the treatment of being overweight, 61% (N=8) of institutions prescribed to overweight-patients a low caloric diet and 78% (N=10) prescribed exercise. A low caloric diet may set patients back into the anorexic-mode of eating and be harmful.

5.2.3.2. Nutritional advice

All the institutions provided nutritional advice to the patient and parents and calculated food energy requirement of patients. In 69% (N=9) of institutions the dieticians were in charge of providing nutritional advice to the patients; in 31% (N=4) of institutions the clinicians offered advice; and in 23% (N=3) the medical doctors specialising in nutrition also gave advice.

The assumption that the calculation of the patient's energy and macronutrient requirement only took place in those institutions working with dietitians was not established. One possible explanation for this lack of association is that in Argentina many institutions include medical doctors specialising in nutrition, who are highly capable of assisting patients nutritionally along the treatment and providing an adequate dietary plan to patients.

Of the 13 institutions, only 10 (77%) calculated the macronutrient requirement of the patients. Patients were advised to consume a mean carbohydrate percentage from the total energy intake of 54% (SD: 5), 18% (SD: 6.2) of protein and 27% (SD: 3) of fat. The number of main meals provided on admission and during treatment were 4.0 and the number of snacks on admission was 1, SD: 1 and, during treatment, 2 (SD: 1). The hypothesis that treatment for AN did not address potential risk factors of chronic diseases such as high percentage of saturated fat from total energy intake is, therefore, rejected. This is supported by the fact that the majority of the institutions calculated macronutrient intake, and fat intake was shown to be less than the recommendation of 30 % of total energy intake (WHO 1990).

5.2.3.3. Nutrient supplementation

Nutrient supplementation was used by most of the institutions; 92% (N=12) prescribed caloric supplements, 77% (N=10) provided vitamins and mineral supplements and 61% (N=8) supplemented food to achieve high energy density (for example adding extra cream, cheese, full fat milk powder, etc.). The assumption that a high fat dietary plan was prescribed to patients to rapidly recover weight loss was, therefore, proved, since more than half the institutions declared that food was supplemented with fat rich food to encourage weight gain. However, this contradicts the point mentioned above that fat intake was 27% of total energy intake. This begs the question: how did most of the institutions manage to prescribe desirable values of fat intake using foods containing high amounts of fat?

All the institutions provided portion size advice on the intake of meat and dairy products. The majority of the institutions (92%, N=12) provided advice on the consumption of bread and cereals, fruit and vegetables and vegetable oils. Advice on the intake of food containing sugar was given by 69% (N=9) of institutions, on salt intake by 85% (N=11), on diet products by 77% (N=10) and on fat rich food by 46% (N=6). If little nutritional counselling and supervision are given in relation to the consumption of fat rich food, patients may perceive this to mean that they could eat this type of food freely. The excessive intake of these food items may increase the future risk of being overweight and cardiovascular disease.

5.2.3.4. Management of risk factors for osteoporosis and cardiovascular disease

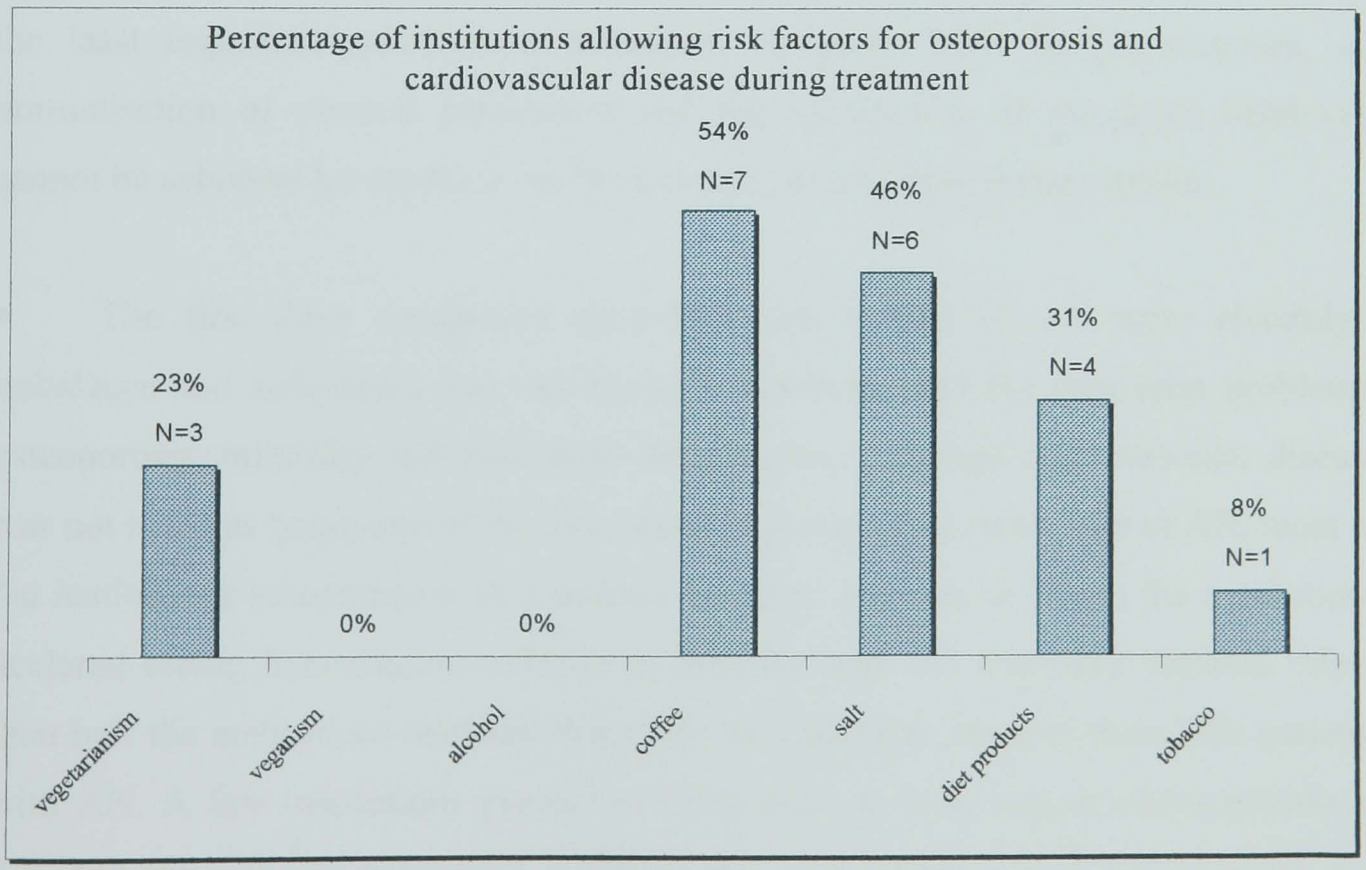
Low intensity-exercise was allowed by all the institutions once patients achieved a safe body weight. Graph 5.1 shows the percentage of institutions allowing risk behavioural patterns in patients with AN. The hypothesis that treatment for AN do not address the potential risk factors for chronic diseases: tobacco smoking and coffee drinking can be partially accepted. More than half the institutions allowed the use of coffee and some of them allowed tobacco to be consumed among patients. Alcohol intake was not allowed during treatment by any institution.

Less than half of the institutions gave advice on salt consumption. Although salt intake should be controlled since many patients used excessive amounts of it to make low calorie foods tastier (Morgan and Lacey 1998). Salt abuse could contribute to the development of hypertension and cardiovascular disease (Law et al 1991; He and Whelton 1997).

The use of diet products (low-calorie foods) was permitted by a third of the institutions. These types of food are controversial in the treatment of AN, since they are linked to energy intake reduction and weight loss.

Veganism (avoidance of animal food) was not allowed, however, other types of vegetarianism were accepted by some institutions (23%). Vegetarianism is related to low body weight and energy intake (Hebbelinck et al 1999), low body mineral densitometry and risk of fractures (Barr et al 1998) and, therefore, to support such food choice may be controversial or detrimental in the treatment of AN.

Graph 5.1



5.3. Discussion

5.3.1. Priorities of treatment

Powers (1984) outlined the indications for admitting patients with AN to hospital. These are:

- Weight loss greater than 30% usual weight over 3 months
- Severe metabolic disturbances (pulse <40 min, temperature <36° C, systolic blood pressure <70 mm Hg, serum potassium <2.5 mmol/l and blood urea >30 mg/dl)
- Severe depression and risk of suicide
- Severe bingeing and purging
- A one-month history of failure to gain weight during outpatient treatment.

The above indications are similar to those which the institutions estimated as being the most important priorities in the treatment of patients with AN. These were to normalise clinical parameters, to eliminate abnormal eating behaviour such as vomiting, diuretic and laxative use, etc. and to eliminate substance use, such as marijuana and cocaine. The promotion of a balanced diet was reported to be one of the least important priorities of treatment. However, body weight recovery, the normalisation of clinical parameters and the elimination of purgative behaviour cannot be achieved by anything but the planning of a healthy dietary intake.

The first three recognised short-term risk factors of AN were electrolyte imbalance and arrhythmia and nutritional deficiencies, and the long-term problems, osteoporosis, infertility and nutritional deficiencies. Although cardiovascular disease was not listed as being one of the three most important long-term risks of AN, most of the institutions recognised it as a serious problem. The rate at which the institutions declared seeing hypercholesterolemia in patients with AN was very variable. More than half the institutions declared that this was a problem in more than 25% patients with AN. A few institutions prescribed medication to treat hypercholesterolemia in AN, and the majority prescribed a dietary plan and exercise, once body weight was healthy. Therefore, it is possible to conclude that most of the institutions were aware

of hypercholesterolemia in the disorder and recognised cardiovascular disease as a problem.

Osteopenia (decreased bone mineral density) is one of the main complications in patients with AN and the following have been suggested for the treatment of this condition: weight gain, treating depressive symptoms, calcium supplementation and normalisation of gonadal function (Carmichael and Carmichael 1995). 1500 mg of calcium is recommended (Powers 1999), of which at least 1000 mg can be provided by food rich in calcium and 500 mg by supplementation (Troter 1997). However, it has been proved that the potential to improve bone mass lost during the active phase of the illness is limited (Powers 1999).

In this study, all the institutions reported that bone abnormalities were a serious complication and prescribed an adequate dietary plan to treat bone problems. However, half the institutions reported that bone loss was a problem in less than 25% of the patients, when they were asked to rate how frequent this complication was. In contrast to this finding, Grinspoon and co-workers (1997) demonstrated that osteoporosis was present in over half of all the patients with AN. Howard and colleagues (1999) observed that 62% patients with AN on admission had osteopenia (bone density between 1 or 2 SD below maximum expected for sex-matched healthy subjects) and 33% had osteoporosis (bone density more than 2 SD below normal). Disagreement between studies may be a result of the lack of availability of bone mass densitometry equipment in many institutions treating AN in Argentina and many more patients than estimated might have had bone loss without this being assessed due to economic restrictions.

5.3.2. Weight management

Most of the institutions set a desirable weight for the patients at some point of the treatment. More than half the institutions declared that being overweight was a problem after re-feeding and this was generally treated with a low calorie dietary plan and exercise prescription. That patients become overweight after refeeding is an alarming finding, but some of the responses to such questions posed might not have

been specific to AN but to other eating disorders. That patients with AN became overweight after re-feeding has serious future implications. Pre-morbid overweight problems are sometimes the case in patients who have had AN (Anderson 1990; Shinder and Sheohard 1993), which suggests that many patients had already been weight cycling before the presentation of the illness. It has been suggested that weight cycling increases visceral fat deposits, changes energy metabolism, causes adverse psychological effects, and increases risk of insulin resistance, hyperlipoproteinemia, diabetes and cardiovascular disease (Atkinson and Stern 1997; Brownell and Rodin 1994). However, evidence on weight cycling has been controversial (National Task Force 1994) and further research in this field is needed.

Patients with restricting AN may have more difficulty in maintaining their weight, because of the lack of motivation to eat and because of a shift in metabolism. Mickley (1999) called this phenomenon hibernation metabolism. If an individual eats less than the minimum intake for an adult (1200 to 1500 Kcal), this causes a decrease in metabolic rate. The body gets used to this small amount of energy and the metabolism slows down, less energy is required for vital body functions and to maintain body weight. Patients with restricting type AN require greater EI than non-restricting AN patients to maintain body weight. By contrast, patients with non-restricting type AN will tend to increase their weight rapidly at the risk of becoming obese (Kaye et al 1986). Patients who have previously been obese gain weight more readily on the same energy intake than patients who have never been overweight (Stordy et al 1977).

An approach in terms of body weight has been to help patients to accept their pre-morbid body weight as natural (Crisp 1970). However, pre-morbid overweight and obesity have been described in patients with AN, especially in non-restricting type of patients, and obesity is also more common in their families (Garner et al 1993; Crisp 1984). Nova and colleagues (2001) observed that patients with AN, who at follow up, had the highest body fat levels were more likely to turn back to the restricting food habits, probably as a reaction to a dissatisfied body perception. Besides, an unhealthy body weight is not compatible with prevention of cardiovascular disease and other chronic diseases. The treatment of the eating disorder

should aim to help patients to achieve a reasonable and stable healthy weight. Weight recovery should be strictly controlled, avoiding weight fluctuations or excessive weight gain, during treatment.

5.3.3. Nutritional advice

Touyz and Beumont (1985), based on their clinical experience, suggested that dietitians should avoid advising about the prevention of obesity, diabetes and arteriosclerosis, as this could reinforce some erroneous ideas that patients may have picked up from the media. The authors pointed out that there is a danger that patients may become dependent on the dietitian as someone to talk to endlessly about food without improving their eating habits and body weight. However, the present thesis shows that patients with AN have increased risk of cardiovascular disease, therefore advice about the prevention of future chronic diseases should be given when patients are psychologically capable of understanding the message. Besides, most of the patients with AN need to acquire healthy eating habits and the nutritional guidelines for healthy people are based on the prevention of future chronic diseases. The fact that more than half the institutions in this study included dietitians or medical doctors specialising in nutrition, as part of the eating disorder team, also supports this argument.

5.3.4. Nutrient supplementation

Psychiatrists are generally pressured, due to economic reasons, to transfer patients with AN from inpatient clinics to less intense treatment (Howard et al 1999). Since weight correction is one of the discharge criteria, patients are usually fed with high-density food to rapidly gain weight. High caloric drinks and high-density food items such as double cream, full fat milk and cheese are used by more than half the institutions to guarantee that patients achieved a high calorie intake. Based on his experience, Russell (1998) recommended the use of high-fat food and less frequent meals with the aim of making it more comfortable for the patient. Imbierowicz and colleagues (2002) compared two dietary treatments for AN: one included a standard diet, and the other included a standard diet plus food supplementation (liquid formula

of 1500 kcal). The patients who received food supplementation gained weight more rapidly and had shorter treatment duration. The authors concluded that food substitution should be integral part of the treatment for AN.

To allow nutrient supplementation to continue indefinitely may be inappropriate, as patients need to be encouraged to eat normal solid food. Besides, the high consumption of food containing saturated fat predisposes to lipid elevation and cardiovascular disease (Hu et al 1997). Nor would the inadequate use of high-fat products help patients who usually suffer from increased blood cholesterol levels. In these situations mono and polyunsaturated oils may be helpful to decrease blood lipids, and frequent fish consumption to improve endothelial function (Sanders 1993).

Fat intake is recommended to represent no more than 30% of total EI even when patients are extremely underweight. In disagreement with the comment of Trotter (1997) in her review on the dietary treatment of eating disorders, it is not eating more fat that will help patients to reduce cholesterol levels, but a varied and balanced diet. The prescription of food containing fat to rapidly achieve weight gain is, therefore, to be restrained. High carbohydrate drinks, consumed with main meals to prevent caries, are an alternative method of increasing EI. High carbohydrate intake may also help to decrease the delayed gastric emptying, which occurs in AN (Robinson and McHugh 1995). Diet therapy for AN should not only address the low body weight of patients, but also its risk factors. At long-term, once patients correct eating problems it is more recommendable to advise the use of low fat dairy products to combat potential risk for cardiovascular disease. There is also a psychological effect, which supports the idea of 'sensible' low fat intake, which patients had adhered to albeit excessively.

The message to patients with AN to facilitate the reduction of their increased cardiovascular risk is to eat a varied diet, including moderate amounts of fat (no more than 30% of total fat intake). To prevent the risk of relapse, vegan and vegetarian diets are to be discouraged, placing the focus on teaching patients to eat an omnivorous and varied diet. With this dietary advice there is no need for vitamin and mineral

supplementation, or for supplementing meals with high-density items to achieve weight gain.

Most of the institutions prescribed vitamin and mineral supplements to patients with AN. The use of vitamin and mineral supplementation is controversial in the treatment of AN; while some authorities recommend their use (Rock and Yager 1987), others claim that this is not necessary, as weight normalisation corrects any medical problem.

5.3.5. Management of risk factors for osteoporosis and cardiovascular disease

Garfinkel and Garner (1982), based on their experience, suggested that the patient's foods do not need to be treated as special, since usually the patient's diet was considered different from the other members of the family. Many patients follow vegan diets or varied forms of vegetarianism (O'Connor et al 1987; Hadigan et al 2000). O'Connor and colleagues (1987) reported that the avoidance of red meat in adolescents should alert families to the possibility of an eating disorder. The downside of some vegetarian diets is that they may predispose to nutritional deficiency, such as iron deficiency and low calcium intake, if vegan, and may be disadvantageous to the nutritional recovery of patients. It may be beneficial for the patients to eat a variety of food and to get use to eating 'forbidden food' such as meat. Any type of vegetarianism reinforces patients' ideas of dieting, food selection and restraint. In contrast, Rock and Yager (1987), in the light of their clinical work with eating disorder patients, suggested that lacto-ovovegetarian diets in patients with eating disorders might be tolerated as long as the diet is balanced. In the present study vegetarianism was tolerated by a quarter of the institutions, while veganism was not allowed during treatment.

How to consider the patient's human right to opt for a vegan/vegetarian diet? Allowing patients to become vegan is like encouraging them to become anorexic. Patients may benefit from knowing that these dietary choices do not help with their recovery and they may be driven by their 'anorexic thinking' to reduce food intake.

Also, to plan a successful and healthy vegan/vegetarian diet requires discipline, organisation and motivation with food, which are characteristics that these patients usually lack. Food dominates patients' thoughts and asking them to plan in advance a healthy combination of food and adequate portions of vegetable intake could be detrimental. In extreme circumstances, if a patient has an ethical and psychological determination to sustain vegetarianism, a diet, which includes nuts, seeds, grains, pulses and dairy products, can provide the necessary nutrients to maintain optimal nutrition. There is not research evidence on the nutritional recovery of vegetarian patients compared with non-vegetarian and not enough research on the nutritional treatment of eating disorders (NICE, 2004).

Low calorie products are to be eliminated on the initial phase of therapy because they are used to exacerbate weight loss and consolidate patients' ideas of dieting and restricting food intake, according to the opinion of practitioners working with eating disorders patients (Toutz and Beumont 1985). Low calorie products also allow patients to think they would be able to eat excessively because they will not put on weight. These products reduce satiety, which is only satisfied by eating more food than would have been consumed (Foltin et al 1988), and predispose patients to subsequent binge eating and purging behaviour. In this study, nearly a third of institutions tolerated the use of low calorie products.

Although excessive caffeine use and coffee intoxication has been reported in patients with AN (Sours 1983 and Forman et al 1997), more than half the institutions agreed with the intake of coffee. Excessive alcohol drinking and tobacco smoking are often other choices of patients, especially the non-restricting type of patients (Goldbloom et al 1992; Haug et al 2001; Crisp 1999). Tobacco smoking is used by many patients as a means of appetite suppression and weight. In the present study, alcohol drinking was not permitted by any institution, though a minority agreed to the use of tobacco. These dietary and behavioural choices do not help to the process of recovery.

5.4. Limitations and recommendations

Half the institutions treating eating disorders in Buenos Aires, which were invited, agreed to take part in this study. Lack of interest or time was the main reason for refusal to participate. The greatest limitation of this study is, therefore, the small sample size. The validity and reliability of the questionnaire has not been assessed, which is in its self-a limitation. There were also biases in the selection of the institutions and the nature of the questionnaire because there might have been different interpretations of the questions by different professionals. Also, dietitians and clinicians filled in the questionnaire and this would not account for the views of the other members of the team. An alternative method of carrying out this study would have been a direct observation of the services, and interviews with all the members of the team.

There is plenty of available evidence on the diet of patients with AN before attending treatment. A study carried out by Herzog and colleagues (1992) demonstrated that fewer than 50% of professionals working with eating disorder patients believed that there was a consensus regarding the treatment of eating disorders. This also applies to the dietary treatment. Further studies in the dietary treatment of patients with AN are necessary in order to obtain improved guidelines for diet therapy. Most of the guidelines for the dietary treatment are based on the clinical experience of experts on the field without scientific evidence. Studies on the effectiveness of different dietary treatments, the appraisal of the dietitian's role in the eating disorder team, the estimation of dietary plans to prevent chronic diseases and the nutritional recovery of vegetarian patients are limited or non-existent.

5.5. Conclusion

Institutions treating eating disorders were questioned, with the aim of investigating the advice they gave with regard to the potential development of future chronic diseases such as cardiovascular diseases and osteoporosis. Most of the institutions recognised cardiovascular disease and osteoporosis as important complications in patients with AN. However, cardiovascular disease was not rated to

be one of the main long-term health problems in the illness. Hypercholesterolemia was estimated to be present in nearly half of all patients and bone abnormalities in a quarter of patients.

Many institutions in the present study were using high fat food to rapidly achieve weight gain. A high fat diet may increase the patient's probability of acquiring incorrect eating patterns, becoming over-weighted or developing heart problems. The current goal seems to be to feed patients to an acceptable weight without taking into account the quality of food intake. The dietary treatment for AN should take into account the prevention of cardiovascular disease and osteoporosis, which patients are susceptible to suffer.

Patients with AN usually start dieting by depriving themselves of certain food because they perceive they are over-weight. They may successfully lose body weight but become amenorrhoeic. Eventually, they may follow a treatment plan and recover the weight lost. They may then relapse and lose all the weight gained. The shift from a low body weight to a high body weight may bring serious long-term health problems. Being overweight after re-feeding was rated as a problem in patients with AN and, therefore, weight gain and eating habits in patients, especially of the non-restricting type, is to be closely supervised until the end of treatment. Since it has been documented that patients continue to eat abnormally at long-term follow up (Morgan and Russell 1975; Eckert et al 1995), dietitians have an essential role in monitoring patients up until the end of the treatment and evaluating whether recovered patients have adequate food habits and body weight.

Chapter 6 - Conclusions

Chapter 6 - Conclusions

The purpose of this thesis was to examine dietary and behavioural patterns in young women with reference to eating disorders and possible future chronic diseases. It also aimed to study risk factors for cardiovascular disease in patients with AN and to elucidate whether treatments for AN, besides treating the evident symptoms of the illness, address this potential risk. Four studies were carried out to consider these issues and the main conclusions for each of them are drawn below. Detailed conclusions were provided at the end of each chapter describing the study.

6.1. Risk factors for osteoporosis and cardiovascular disease in adolescent women

Dietary inadequacies, in contrast with what had been expected, were more frequent in the upper-income schoolgirls, who showed a lower energy and fibre intake. Behavioural patterns associated with cardiovascular disease, such as lack of exercise, tobacco and alcohol use were more prevalent in the low-income group. A high percentage of the upper socio-economic girls were underweight and preoccupied with body weight and dieting, perhaps due to a higher social pressure to achieve thinness. Girls with the highest BMI were more likely to diet to lose weight. Therefore, based on the results of this study, preventive campaigns addressing the adoption of unhealthy behavioural patterns to lose weight (for example alcohol, tobacco and coffee use) would be more appropriate if targeted at the low-income groups. While preventive campaigns focusing on body dissatisfaction and dieting would be directed at the heaviest girls and the upper-income groups

Body dissatisfaction in teenagers often leads to dieting. Girls restrict their food intake or engage in inappropriate dietary and behavioural patterns to lose weight, which may have the short-term consequence of increasing their risk for eating disorders, and the long-term consequences of potential cardiovascular disease and osteoporosis. The following studies examine whether patients with AN also exhibit an increased risk of cardiovascular disease.

6.2. Clinical notes review

TC, LDL and HDL concentrations were higher in patients with AN compared with Argentinean subjects of healthy weight. The elevated levels of TC decreased after an average of nine months of treatment. This study added valuable information to the existing literature on hypercholesterolemia in AN because it had a considerably larger sample size than previous cholesterol studies. Also, it is one of the first clinical assessments on Argentinean patients and one of the most contemporary studies on healthy young Argentinean females. Since it has been proved by this study that hypercholesterolemia is a problem in AN, it justified the need for the next study, which assessed cholesterol concentrations and investigated possible causes of hypercholesterolemia in a group of patients with AN.

6.3. Follow up patients with AN

TC, LDL, Apo B and fibrinogen concentrations, were elevated in patients on admission compared with controls, while retinol and tocopherol concentrations were decreased. Deficiencies in T3, T4 and estradiol were likely to be responsible for the increased TC values. These results suggested that these patients were more susceptible to oxidative damage and to morbidity and mortality from cardiovascular disease. After four months of treatment, the low BMI improved and there was a tendency for most of the altered markers to normalise. As the previous study demonstrated, a longer follow-up period is required to observe significant changes. HDL levels decreased significantly on the follow up, leaving patients with an undesirable lipid profile.

Cardiovascular disease is not commonly a problem in these patients when studied because they are generally young and age-protected. As patients, without appropriate treatment, get older this risk may increase, as they may become more susceptible to cardiovascular disease. This study provided original evidence on cardiovascular risk in AN and it is one of the few biochemical studies in Argentinean

patients with AN. The next study evaluated whether treatment programs for AN deal with the apparent greater risk for cardiovascular disease.

6.4. Treatment assessment

The treatment institutions considered osteoporosis but not cardiovascular disease to be among the main long-term health concerns of AN. Hypercholesterolemia was estimated to be present in nearly half, and bone abnormalities in a quarter of all patients. 60% of the institutions prescribed a high fat diet to rapidly achieve weight correction. This may increase patients' probability of acquiring incorrect eating patterns, developing heart problems or becoming over-weight, which was appraised as a problem by several institutions.

The institutions took arbitrary decisions in the allowance of dietary and behavioural choices contributing to chronic diseases (for example, coffee, alcohol and smoking). These choices have a potential to maintain eating disorder behaviour and may also have harmful consequences on the course of osteoporosis and cardiovascular disease. The current dietary treatments for AN also need to focus on the prevention of these chronic diseases, which patients are more susceptible to suffer.

Overall conclusion

This thesis has demonstrated that 62% of schoolgirls wanted to lose weight, and that 2.5% have been diagnosed as having an eating disorder. These findings portray a young Argentinean population overly concerned with body weight that is comparable to more developed societies. The possible outcomes of young women dieting and engaging in unhealthy weight-reducing behaviour are eating disorders, cardiovascular disease and osteoporosis.

References

- American College of Sport Medicine (1985) 'Guidelines for graded exercise testing and exercise prescription', 3rd ed., Philadelphia, Lea and Febinger.
- American College of Sports Medicine (1995), 42nd Meeting, Minneapolis, Minnesota, Medic Science Sports Exercise, 27, 5, Supp.1-250.
- American Psychiatric Association (1994) 'Diagnostic and Statistical Manual of Mental Disorders', 4t, 731, Washington APA.
- Ames R (1983) ´Negative effects of diuretic drugs on metabolic risk factors for coronary heart diseases: possible alternative drug therapies´, Am J Cardiol, 24, 51, 632-8.
- Anderson A (1990) 'Diagnosis and treatment of males with eating disorders' in Anderson A (ed.) 'Males with eating disorders', Brunnel/Mazel, New York.
- Anyan W (1974) 'Changes in erythrocyte sedimentation rate and fibrinogen during anorexia nervosa´', J Pediatr, 85, 4, 525-7
- Arden MR, Weiselberg EC, Nussbaum MP, Shenker IR, Jacobson MS (1990) 'Effect of Weight Restoration on the Dyslipoproteinemia of Anorexia Nervosa', J Adoles Health Care, 11, 199-202.
- Armstrong N, Balding J, Gentle P, Kirby B (1990) 'Patterns of physical activity among 11 to 16 year old British children', Brit Med J, 28, 301, 6745, 203-5.
- Arnold LM, Ball MJ, Duncan AW, Mann J (1993) 'Effect of isoenergentic intake of three or nine meals on plasma lipoproteins and glucose metabolism', Am J Clin Nut, 57, 446-51.
- Ashwell M (ed.) (1997) 'Diet and Heart disease', 2nd ed., Chapman and Hall, London.
- Atkinson R and Stern J (1997) 'Weight cycling. Definitions, Mechanisms and Problems with Interpretetion', in Bray G. et al (eds.) 'Handbook of obesity', Marcel Dekker, Inc., New York.
- Avery D (1998) 'The bridgenews forum: viewpoints on farming policy and agricultural issues', 1st May http://www.foodstuff.org (assessed 14th December 2002)
- Bachard LK, Guido D, Katzman D, Litt IF, Marcus R. (1990) 'Decreased bone density in adolescent girls with anorexia nervosa', Pediatrics, 86, 3, 440-7.

- Balding J (1999) 'Young people in 1998 and looking back as far as 1983', School Education Unit, London. Bandini L, Must A, Cyr H, Anderson S, Spadano J, Dietz W. (2003) 'Longitudinal changes in the accuracy of reported energy intake in girls 10-15 y of age', Am J Clin Nutr, 78, 480-4
- Bannai C, Kuzuya N, Koide Y, Fujita T, Itakura M, Kawai K, Yamashita K (1988) 'Assessment of the relationship between thyroid hormone levels and peripheral metabolism in patients with anorexia nervosa', Endocrinol Jpn, 35, 3, 455-62.
- Barber SA, Bull NL, Buss DH (1985) 'Low iron intakes among young women in Britain', Brit Med J, 9, 290, 743-4.
- Barker M (1989) 'Diet, lifestyle and health in Northern Ireland', Coleraine, Centre for Applied Health Studies, University of Ulster.
- Barker D (1993) 'Fetal origins of coronary heart disease', Brit Heart J, 69, 195-6.
- Barker D (1995) 'Fetal origins of coronary heart disease', Brit Med J, 311, 171-4.
- Barker DJ, Bull AR, Osmond C, Simmonds SJ (1990) 'Fetal and placental size and risk of hypertension in adult life', BMJ, 301, 259-62.
- Barr SI, Prior JC, Janelle KC, Lentle B (1995) 'Dieting attitudes and behaviour in urban high school students. Implications for calcium intake', J Adolescent Health, 16, 458-64.
- Barr S et al (1998) 'Spinal bone mineral densitometry in premenopausal vegetarian and nonvegetarian women: Cross-sectional and prospective study', J Am Diet Assoc, 98, 76-85.
- Bazan M (2001) 'Proyecto de investigacion de la niñez y obesidad'. Childhood obesity, Buenos Aires. www.nutrinfo.com.ar/pagina/info/pinocho.html (assessed 15th October 2002).
- Beaumont PJ, Chambers TL, Rouse L, Abraham SF (1981) 'The diet composition and nutritional knowledge of patients with anorexia nervosa', J Human Nutrition, 35, 265-73.
- Beardsworth A and Keil E (1993) 'Contemporary vegetarianism in the U.K.: challenge and incorporation? ', Appetite, 20, 3, 229-34.
- Beevers G, Lip GYH, O'Brien E. (2001) 'Blood pressure measurement. Part I-Shygmomanometry: factors common to all techniques', Brit Med J, 322, 21, 981-5.
- Beier RC (1984) 'Homocysteine and arteriosclerosis', Nature, 310, 18.

- Bello M (1995) 'Prevalence of eating disorders in a school population in Buenos Aires, Argentina', ECED, Doublin.
- Benthin A, Slovic P, Severson H (1993) 'Psychometric study of adolescent risk perception', J Adolescence 16, 2, 153, 168.
- Bhanji S and Mattingli D (1981) 'Anorexia nervosa: some observations on "dieters" and "vomiters", cholesterol and carotene', Brit J Psychiatry, 139, 238-41.
- Biller BM, Saie V, Herzog DB, Rosenthal DI, Holzman S, Klibanski A. (1989) 'Mechanisms of osteoporosis in adult and adolescent women with anorexia nervosa', J Clin Inv Met, 68, 3, 548-54.
- Black AE, Goldberg GR, Jebb SA, Livingstone MB, Cole TJ, Prentice AM (1991) 'Critical evaluation of energy intake data using fundamental principles of energy physiology: 2. Evaluating the results of published surveys', Eur J Clin Nutr, 45, 583-99
- Blendis L and Crisp A (1968) 'Serum cholesterol levels in anorexia nervosa', Postgrad Med J, 44, 510, 327-30
- Blyth D, Simmons RG, Zakin DF (1985) 'Satisfaction with body image for early adolescent female: the impact of pubertal timing within different school environments', J Youth Adolescent, 14, 3, 207-25.
- Boland B, Beguin C, Zech F, Desager JP, Lambert M (2001) 'Serum beta-carotene in anorexia nervosa patients: a case control study', Int J Eat Disord, 30, 3, 299.
- Bolling K (1993) 'Smoking among secondary school children in England in 1993', Social Survey Division of OPCS, London, HMSO.
- Boushey CJ, Beresford SA, Omenn GS, Motulsky AG (1995) 'A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intake', J Am Med Assoc, 274, 1049-57.
- Brannen J, Dodd K, Oakley A, Storey P (1994) 'Young people, health and family life', Open Univeristy Press, Buckingham.
- Brattstrom LE, Hardebo JE, Hultberg BL (1984) 'Plasma homocysteine in women on oral oestrogen containing contraceptives and in men with oestrogen treated prostatic carcinoma', Scand J Clin Lab Invest, 52, 283-7.
- Brattstrom L and Wilcken D (2000) 'Homocysteine and cardiovascular disease: cause or effect', Am J Clin Nutr, 72, 315-23.

- Brenner R (1981) 'Nutritional and hormonal factors influencing desaturation of essential fatty acids', Prog Lipid Res, 20, 41-7.
- Brinch M, Isager T, Tolstrup K (1988) 'Anorexia Nervosa and motherhood: reproduction pattern and mothering behaviour of 50 women', Acta Psychiatr Scand, 77, 5, 611-7.
- Brown SL, Salive ME, Harris TB, Simonsick EM, Guralnik JM, Kohout FJ (1994) 'Low cholesterol concentrations and severe depressive symptoms in elderly people', Brit Med J, 308, 6940, 1328-32.
- Brownell K and Rodin J (1994) 'Medical, metabolic and psychological effects to weight cycling', Arch Int Med, 27, 154, 1325-30.
- Brunch H (1962) 'Perceptual and conceptual disturbances in anorexia nervosa', Psychosomatic Medicine, 24, 187-94.
- Brunner E, Stallone D, Juneja M, Bingham S, Marmot M (2001) 'Dietary assessment in the Whitehall II: comparison of the 7 d diet diary and food-frequency questionnaires', Brit Med J, 86, 405-11.
-Button E (1990) 'Eating Disorders. Personal Construct, Therapy and Change', London, Willey.
- Campbell K, Waters E, O'Meara S, Kelly S, Summerbell C Interventions for preventing obesity in children (Cochrane Review). In: The Cochrane Library, Issue 2, 2004. Chichester, UK: John Wiley & Sons, Ltd.
- Cancer Prevention Group (1994) 'The effect of Vitamin E and Beta carotene on the incidence of lung cancer and other cancers in male smokers', New Eng Med J, 330, 1029-35.
- Carmichael K and Carmichael D (1995) 'Bone metabolism and osteopenia in eating disorders', Medicine (Baltimore), 74, 5, 254-67.
- Cravo ML, Gloria LM, Selhub J, Nadeau MR, Camilo ME, Resende MP, Cardoso JN,
- Casanueva FF, Borras CG, Burguera B, Muruais C, Fernandez M, Devesa J (1987) 'Steroid and neuroendocrine function in anorexia nervosa', J Steroid Biochemistry, 27, 1-3.
- Case T, Lemieux S, Kennedy SH, Lewis GF (1999) 'Elevated plasma lipids in patients with binge eating disorders are found only in those who are anorexic', Int J Eat Disord, 25, 187-93.

- Casper RC, Kirschner B, Sandstead HH, Jacob RA, Davis JM (1980) 'An evaluation of trace metals, vitamins, and taste function in anorexia nervosa', Am J Clin Nutr, 33, 8, 1801-8
- Casper RC, Pandy GN, Jaspan JB, Rubenstein AH (1988a) 'Hormone and metabolite plasma levels after oral glucose in bulimia and healthy controls', Biol Psych, 24, 663-74.
- Casper RC, Pandey G, Jaspan JB, Rubenstein AH (1988b) 'Eating attitudes and glucose tolerance in anorexia nervosa patients at 8-year follow up compared to control subjects', Psychiatric Research, 25, 283-99.
- Chikasue F, Kojima T, Miyazaki T, Yashiki M (1988) 'A medicolegal case of anorexia nervosa', Hiroshima J Med Science, 37, 157-60.
- Christopher K, Tammaro D, Wing EJ (2000) 'Early scurvy complicating anorexia nervosa', South Med J, 95, 9, 1065-6.
- Cohen H (1958) 'Observations on carotenemia', Ann Int Med, 48, 219.
- Colao A, Pivonello R, Spiezia S, Faggiano A, Ferone D, Filippella M, Marzullo P, Cerbone G, Siciliani M, Lombardi G (1999) 'Persistence of increased cardiovascular risk in patients with Cushing's disease after 5 years of successful cure', J Clin End Met, 84, 2664-72.
- Collins N (1996) 'The investigation of predictors and characteristics associated with body size dissatisfaction in female adolescents', Thesis, Institute of Psychiatry, University London.
- Collins R et al (2002) 'The MRC/BHF Heart Protection Study: preliminary results', Int J Clin Pract, 56, 1, 53-6.
- Coniglio R et al (1992) 'Prevalencia de los factores de riesgo para la aterosclerosis coronaria en zonas urbanas de la Patagonia Argentina', Medicina (BA), 52, 320-2.
- Coniglio R et al (1997) 'Estudio de adolescentes de factores lipoproteicos para la aterosclerosis silos y asociados con incrementos de Lp(a)', IV Congreso Nacional de Bioquímica (Cubra IV), Tucuman-Argentina.
- Cook J et al (1973) 'A survey of the nutrition status of school children', Brit J Preventive Social Medicine, 27, 91-9.

- Crawley H (1993) 'The energy, nutrient and food intakes of teenagers aged 16/17 years in Britain. 1. Energy. macronutrients and non-starch polysaccharides', Brit J Nutr, 70, 1, 15-26.
- Cremerius J (1978) 'Zur Prognose der Anorexia Nervosa (11 Sech Sundzwanzing bis neunundzwanzigjahrige kalamnesen psychotherapeutisch unbehandetter Falle)', Zeitschriftfur Psychosomatishe Medizin und Psychoanalyse, 178, 24, 56-69.
- Crisp A (1965) 'Some aspects of evolution, presentation and follow-up of Anorexia Nervosa', Proc Royal Soc Med, 58, 10, 814-20.
- Crisp A (1967) 'Insulin response to a rapid intravenous injection of dextrose in patients with Anorexia Nervosa and obesity', Post Med J, 43, 97, 102.
- Crisp AH, Blendis LM, Pawan GL (1968) 'Aspects of Fat Metabolism in Anorexia Nervosa', Metabolism; 17; 12; 1109-18.
- Crisp A (1970) 'Premorbid factors in adult disorders of weight, with particular reference to primary anorexia nervosa (weight phobia). A literature review' , J Psychosomatic Research, 14, 1-22.
- Crisp AH, Palmer RL, Kalucy RS (1976) 'How common is Anorexia Nervosa?', Brit J Psychiatry, 128, 549-54.
- Crisp A (1981) 'Anorexia Nervosa at normal body weight-the abnormal normal weight control syndrome', Int J Psychiatry, 11, 3, 203-33.
- Crisp A (1984) 'The psychopathology of anorexia nervosa: getting the 'heat' out of the system', in Stunkard A and Stellar E. (eds.) 'Eating and its disorders', Raven Press, New York, 209-34.
- Crisp A, Sedgwick P, Halek C, Joughin N, Humphrey H (1999) 'Why may teenage girls persist in smoking?' J Adolescence, 22, 657-72.
- Crisp A and Stonehill E (1967) 'Hypercarotenaemia as a symptom of weight phobia', Postgrad Med J, 43, 505, 721-5.
- Curran-Celentano J, Erdman JW Jr, Nelson RA, Grater SJ (1985) 'Alterations in vitamin A and thyroid hormone status in anorexia nervosa and associated disorders', Brit Med J, 26, 303, 6809, 1019-22.
- Dally P (1954) 'Carotenemia occurring in a case of anorexia nervosa', Brit Med J, 1, 1333.
- Dally P and Sargent W (1966) 'Treatment and outcome of anorexia nervosa', Brit Med J, ii 793-5.

- Department of Health (1989) 'The diet of British Schoolchildren', Report on Health and Social subjects8, 36, London, Stationary Office.
- Deter D and Herzog W (1994), 'Anorexia Nervosa in a Long- Term Perspective: results of the Heidelberg Mannheim Study', Psycosomatic Medicine, 56, 20-27.
- De Simone G, Scalfi L, Galderisi M, Celentano A, Di Biase G, Tammaro P, Garofalo M, Mureddu GF, de Divitiis O, Contaldo F (1994) 'Cardiac abnormalities in young women with anorexia nervosa', Brit Heart J, 71, 287-92.
- Dietary Reference values for food energy and nutrients for the UK (1991), The Stationary Office, London.
- Dixon Z et al (1998) 'The effect of a low carotenoid diet on malondialdehyde-Thiobarbituric Acid concentrations in women: a placebo-controlled double-blind study', J Am Coll Nutrition, 17, 1, 54-8.
- Douyon L and Schteingart D (2002) 'Effects of obesity and starvation on thyroid hormone, growth hormone and cortisol secretion', End Metab Clin North Am, 31, 1, 173-89.
- Dornbusch S et al (1984) 'Sexual maduration, social class, and desire to be thin among adolescent females', J Dev Behaviour Pediatry, 5, 6, 308-14.
- Dreyfuss F and Czaczkes W (1959) 'Blood cholesterol and uric acid of healthy medical students under stress of an examination', Arch Int Medicine, 103, 708-11.
- Drewnowski A, Pierce B, Halmi KA (1988) 'Fat aversion in eating disorders', Appetite, 10, 119-31.
- DSMIV (1994) American Psychiatric Association Diagnostic and Statistical Manual of Mental Disorders. 4t. Washington, APA; p. 731.
- Durnin J and Raham M (1967) 'The assessment of the amount of fat in the human body from measurements of skinfold thinness', Brit J Nutr, 21, 681-9.
- Durnin J and Womersley J (1974) 'Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women 16-72 years', Brit J Nutr, 32, 77.
- Dwyer JT, Dietz WH, Andrews EM, Suskind RM (1982) 'Nutritional status in vegetarian children', Am J Clin Nutr, 204-16.
- Eating Disorders Association (EDA) http://www.edauk.com/default.htm (assessed 24th January 2003).

- Echeverria RF et al (1988) 'Prevalence of arterial hypertension in La Plata, Argentina', Medicina (B Aires), 48, 1, 22-8.
- Eckert ED, Halmi KA, Marchi P, Grove W, Crosby R (1995) 'Ten-year follow-up of anorexia nervosa: clinical course and outcome', Psychological Medicine, 25, 143-156.
- FAO/WHO (1994) 'Fats and oils in human nutrition (Report of a joint expert consultation)' Food and Nutrition Paper 57, Rome, FAO.
- Fapri-Ireland Partnership Economic Projections for Irish Agriculture http://www.tnet.teagasc.ie/fapri/af98/meatconsumptioneu.htm, (assessed 10th October 2002).
- Farkkila MA, Tilvis RS, Miettinen TA (1988) 'Raised plasma cholesterol precursors with gut resection', Gut, 29, 2, 188-95.
- Farrant H (2001) 'Essential Fatty Acids Fat-soluble Vitamins & Cholesterol In Anorexia Nervosa', MSc Nutrition, King's College London.
- Feighner JP, Robins E, Guze SB, Woodruff RA Jr, Winokur G, Munoz R (1972) 'Diagnostic criteria for use in psychiatric research', Arch Gen Psychiatry, 26, 57-63.
- Feillet F, Feillet-Coudray C, Bard JM, Parra HJ, Favre E, Kabuth B, Fruchart JC, Vidailhet M (2000) 'Plasma cholesterol and endogenous cholesterol synthesis during refeeding in anorexia nervosa', Clin Chim, 294, 45-56.
- Feld S and Dickey R. (2001) 'An Association Between Varying Degrees of Hypothyroidism and Hypercholesterolemia in Women: The Thyroid-Cholesterol Connection', Prev Cardiol, 4, 4, 179-182.
- Folsom A (1991) 'Population correlates of plasma fibrinogen and factor VII, putative cardiovascular risk factors', Atherosclerosis, 91, 3, 191-205.
- Folsom AR, Qamhieh HT, Flack JM, Hilner JE, Liu K, Howard BV, Tracy RP (1993) 'Plasma fibrinogen: levels and correlated in young adults. The Coronary Artery Risk Development in Young Adults (CARDIA) Study', Am J Epidemiol, 15, 138, 12, 1023-36.
- Foltin RW, Fischman MW, Emurian CS, Rachlinski JJ (1988) 'Compensation for caloric dilution in humans given unrestricted assess to food in a residential laboratory', Appetite, 10, 1, 13-24.
- Forman J, Aizer A, Young CR (1997) 'Myocardial infarction resulting from caffeine overdoses in an anoretic women', Ann Emerg Med, 29, 1, 178-80.

- Francis K (1979) 'Psychologic correlates of serum indication of stress in man: a longitudinal study', Psychosom Medicine, 41, 617-28.
- Frank RE, Serdula M, Abel GG (1987) 'Bulimic eating behaviours: association with alcohol and tobacco', Am J Public Health, 77, 369-70.
- Frankel EN, Kanner J, German JB, Parks E, Kinsella JE (1993) 'Inhibition of oxidation of human low-density lipoprotein by phenolic substance in red wine', Lancet, 341, 454-57
- Franssila-Kallunki A, Rissanen A, Ekstrand A, Eriksson J, Saloranta C, Widen E, Schalin-Jantti C, Groop L. (1991) 'Fuel metabolism in anorexia nervosa and simple obesity', Metabolism', 40, 7, 689-94.
- Friedman M and Rosenman RH (1959) 'Association of specific behaviour patterns with blood and cardiovascular findings', J Am Med Assoc, 169, 1286-96.
- Friedman M, Rosenman RH, Byers S (1964) 'Changes in serum lipids and capillary circulation after fat ingestion in men exhibiting a behaviour pattern (A) associated with coronary heart disease', Circulation, 28, 724.
- Friewald W et al (1972) 'Estimation of the correlation of LDL cholesterol in plasma, without the use of the preparative ultracentrifuge', Clin Chem, 18, 499-502.
- Frisch R (1990) 'The right weight: body fat, menarche and ovulation', Baill Cli Obstet Gynaecol, 4, 3, 419-39.
- Frusztajer N, Dhuper S, Warren M, Brooks-Gunn J, Fox R. (1990) 'Nutrition and the incidence of stress fractures in ballet dancers', Am J Clin Nutr, 51, 779-83.
- Furman R. et al (1967) Progress in Bioch. Pharm, vol.2
- Furnham A and Alibhai N (1983) 'Cross-cultural differences in the perception of female body shapes', Int J Eat Disord, 13, 829-37.
- Garcia S et al (1997) 'Anorexia y Bulimia, Realidad en el norte y centro de la provincia de Santa Fe', Ambito Farmaceutico, Colegio de Farmaceuticos de Santa Fe, Santa Fe-Argentina, 4-7.
- Garcia-Rubira JC, Hidalgo R, Gomez-Barrado JJ, Romero D, Cruz Fernandez JM (1994) 'Anorexia Nervosa and myocardial infarction', Int J Cardiology, 45, 138-40.
- Garfinkel PE, Moldofsky H, Garner DM (1980) 'The heterogeneity of anorexia nervosa. Bulimia as a distinct group', Arch Gen Psychiatry, 37, 9,1036-40.

- Garfinkel PE and Garner D (1982) 'Anorexia Nervosa a multidimensional perspective', Brunnel/Mazel, New York.
- Garfinkel PE, Brown GM, Stancer HC, Moldofsky H (1975) 'Hypothalamic-pituitary function in anorexia nervosa', Arch Gen Psychiatry, 32, 6, 739-44.
- Garner DM, Garner MV, Rosen LW (1993) 'Anorexia nervosa "restricters" who purge: implications for subtyping anorexia nervosa', Int J Eat Disord, 13, 2, 171-85.
- Garner RM, Friedman BN, Jackson NA (1998) 'Methodological concerns when using silhouettes to measure body image', Perc Motor Skills, 86, 387-95
- Garner RM, Stark T, Jackson NA, Friedman BN (1999) 'Development and validation of two new scales for assessment of body image', Perc Motor Skills, 89, 881-93
- Goddard E (1990) 'Why children start smoking', Office of population censuses and surveys, Social Survey Division, HMSO, London.
- Gold PW, Gwirtsman H, Avgerinos PC, Nieman LK, Gallucci WT, Kaye W, Jimerson D, Ebert M, Rittmaster R, Loriaux DL, et al (1986) 'Abnormal hypothalamic-pituitary-adrenal function in anorexia nervosa; pathophysiologic mechanism in underweight and weight-corrected patients', New Eng J Med, 314, 1335-42.
- Goldberg GR, Black AE, Jebb SA, Cole TJ, Murgatroyd PR, Coward WA, Prentice AM (1991) 'Critical evaluation of energy intake data using fundamental principles of energy physiology: 1 Derivation of cut-off limits to identify under-recording', Eur J Clin Nutr, 45, 12, 569-81.
- Goldbloom DS, Naranjo CA, Bremner KE, Hicks LK (1992) 'Eating disorders and alcohol abuse in women', Brit J Addiction, 87, 6, 913-9.
- Golden NH, Jacobson MS, Schebendach J, Solanto MV, Hertz SM, Shenker IR. (1997) 'Resumption of menses in anorexia nervosa', Arch Pediatr Adolesc Med, 151, 1, 16-21.
- Graham H (1993) 'When life's a drag. Women smoking and disadvantage', Department of Health, London.
- Gregory J et al (1990) 'The dietary and nutritional survey of British adults', Stationary office, London.
- Gregory G et al (2000) 'National diet and nutrition survey: young people aged 4 to 18 years'. Volume I Findings, The Stationary Office, London.

- Grinspoon S, Herzog D, Klibanski A (1997) 'Mechanisms and treatment options for bone loss in anorexia nervosa', Psychopharmacol Bull, 33, 3, 399-404.
- Grinspoon S, Miller K, Coyer C, Krempin J, Amstrong C, Pihs S, Herzog D, Klibanski A. (1999) 'Severity of osteopenia in estrogen deficient woman with anorexia nervosa and hypothalamic amenorrhea', J Clin End Met, 84, 6, 2049-55.
- Gull W (1874) 'Anorexia nervosa (apepsia hysterica, anorexia hysterica)', Transactions of the Clinical Society London, 7, 22-8.
- Gunstone F (1992) 'The lipid handbook', 2nd ed., Chapmans and Hall, London.
- Gwirtsman HE, Kaye WH, Curtis SR, Lyter LM (1989a) 'Energy intake and dietary macronutrient content in women with anorexia nervosa and volunteers', J Am Diet Ass, 89, 1, 54-7
- Gwirtsman HE, Kaye WH, George DT, Jimerson DC, Ebert MH, Gold PW (1989b) 'Central and peripheral ACTH and cortisol levels in anorexia nervosa and bulimia', Arch Gen Psychiatry, 46, 1, 61-9.
- Hadigan CM, Anderson EJ, Miller KK, Hubbard JL, Herzog DB, Klibanski A, Grinspoon SK (2000) 'Assessment of macronutrient and micronutrient intake in women with Anorexia Nervosa', Int J Eat Disord, 28, 284-92.
- Hackett AF, Rugg-Gunn AJ, Appleton DR, Eastoe JE, Jenkins GN (1984) 'A two year longitudinal nutritional survey of 405 Northumberland children initially aged 11.5 years', Brit J Nutr, 51, 67-74.
- Halek C, Kerry S, Humphrey H, Crisp AH, Hughes JM (1993) 'Relationship between smoking, weight and attitudes to weight in adolescent schoolgirls', Am J Clin Nutr, 50, 3, 545-50.
- Hales CN and Randle P (1963) 'Effects of low carbohydrate diet and diabetes mellitus on plasma concentrations of glucose, non-esterified fatty acids and insulin during oral glucose tolerance test', Lancet, I, 790.
- Hales CN, Barker DJ, Clark PM, Cox LJ, Fall C, Osmond C, Winter PD (1994) 'Fetal ad infant growth and impaired glucose tolerance at age 64', Atherosclerosis, 110, Sup. S83-91.
- Halmi K and Fry M (1974) 'Serum Lipids in Anorexia Nervosa', Biological Psychiatry, 8, 2, 159-67.
- Halmi K and Falk J (1981) 'Common Physiological Changes in Anorexia Nervosa', Int J Eat Disord, 1, 16-27.

- Hamsten A, Iselius L, de Faire U, Blomback M (1987) 'Genetic and cultural inheritance of plasma fibrinogen concentration', Lancet, 31, 2, 988-91.
- Harper (2000) 'Harper's biochemistry', XXV ed., New York, McGraw-Hill.
- Haug NA, Heinberg LJ, Guarda AS (2001) 'Cigarette smoking and its relationship to other substance use among eating disordered inpatients', Eat Weight Disord, 6, 3, 130-9.
- Hauger-Kevene J and Balossi E (1987) 'Coronary heart disease mortality and coronary risk factors in Argentina', Cardiology, 74,2, 133-40.
- Hawley R (1985) 'The outcome of anorexia nervosa in younger subjects', British Journal of Psychiatry, 146, 657-60.
- He J and Whelton P (1997) 'Role of sodium reduction in the treatment and prevention of hypertension', Current Opin Cardiol, 12, 202-7.
- Hebbelinck M, Clarys P, De Malsche A (1999) 'Growth, development and physical fitness of Flemish vegetarian children, adolescents and young adults', Eu J Clin Nutr, 70 Sup., 579S-85.
- Heckers H, Gobel U, Kleppel U (1994) 'End of the coffee mystery: diterpene alcohols raise serum LDL and triglycerides', J Int Medicine, 235, 2, 192-3.
- Hegsted D (1986) 'Serum cholesterol response to dietary cholesterol: a re-evaluation', Am J Clin Nutr, 44, 299-305.
- Hernandez-Avila M, Colditz GA, Stampfer MJ, Rosner B, Speizer FE, Willett WC (1991) 'Caffeine, moderate alcohol intake and risk of fractures of hip and forearm in middle-aged women', Am J Clin Nutr, 54, 1, 157-63.
- Herpertz-Dahlmann BM, Wewetzer C, Schulz E, Remschmidt H (1996) 'Course and Outcome in adolescent anorexia nervosa', Int J Eat Disord, 19, 4, 335-45.
- Herzog DB, Keller MB, Lavori PW (1988) 'Outcome in anorexia nervosa and bulimia nervosa. A review of the literature', J Nervous Mental Disease, 176, 3, 131-43.
- Herzog D, Keller M, Strober M, Yeh C, Pai S (1992) 'The current status of treatment for anorexia nervosa and bulimia nervosa', Int J Eat Disord, 12, 215-20.
- Herzog W, Deter HC, Fiehn W, Petzold E (1997) 'Medical findings and predictors of long-term physical outcome in anorexia nervosa: a prospective, 12-year follow-up study', Psychological Medicine, 27, 269-79.

- Hill AJ, Oliver S, Rogers PJ (1992) 'Eating in the adult world: The rise of dieting in childhood and adolescence', Brit J Clin Psychology, 31, 95-105.
- Hladovec J (1979) 'Experimental homocysteinemia, endothelial lesions and thrombosis', Blood Vessels, 16, 202-5.
- Hogg C (1995) 'A guide to purchasing and providing services' Eating Disorder Association, London.
- Hofstetter A et al (1986) 'Increased 24-hour energy expenditure in cigarette smokers', New Eng J Medicine, 314, 79-82.
- Holman RT, Smythe L, Johnson S (1979) 'Effect of sex and age on fatty acid composition of human serum lipids', Am J Clin Nutr, 32, 12, 2390-9.
- Holman RT, Adams CE, Nelson RA, Grater SJ, Jaskiewicz JA, Johnson SB, Erdman JW Jr (1995) 'Patients with anorexia nervosa demonstrate deficiencies of selected essential fatty acids, compensatory changes in nonessential fatty acids and decreased fluidity of plasma lipids', J Nutrition, 125, 4, 901-7.
- Homma Y, Homma K, Iizuka S, Iigaya K (2002) 'A case of anorexia nervosa with severe hyperlipidemia', Int J Eat Disord, 32, 1, 121-4.
- Horwitt MK (1980) 'Interpretation of human requirements for vitamin E', in Machlin L (ed.) 'Vitamin E a comprehensive treatise', Marcel Dekker, New York.
- Hotta M, Shibasaki T, Masuda A, Imaki T, Demura H, Ling N, Shizume K (1986) 'The responses of plasma adrenocorticotrophin and cortisol to corticortrophin-releasing hormone (CRH) and cerebrospinal fluid immunoreactive CRH in Anorexia Nervosa patients', J Clin End Met, 62, 2, 319-24.
- Howard WT, Evans KK, Quintero-Howard CV, Bowers WA, Andersen AE (1999) 'Predictors of success of failure of transition to day hospital treatment for inpatients with anorexia nervosa', Am J Psychiatry, 156,11, 1697-702.
- Hu FB, Stampfer MJ, Manson JE, Rimm E, Colditz GA, Rosner BA, Hennekens CH, Willett WC (1997) 'Dietary fat intake and the risk of coronary heart disease in women', New Engl J Med, 337, 1491-9.
- Hubert HB, Feinleib M, McNamara PM, Castelli WP (1983) 'Obesity as an independant risk facto for cardiovascular disease: a 26-year follow-up of participants in the Framingham Heart Study', Circulation, 67, 5, 968-77.

- Hung CJ, Huang PC, Lu SC, Li YH, Huang HB, Lin BF, Chang SJ, Chou HF (2002) 'Plasma homocysteine levels in Taiwanese vegetarians are higher than those of omnivore', J Nutrition, 132, 2, 152-8.
- Imbierowicz K, Braks K, Jacoby GE, Geiser F, Conrad R, Schilling G, Liedtke R (2002) 'High-caloric supplements in anorexia treatment', Int J Eat Disord, 32, 2, 135-45.
- Imeson JD, Haines AP, Meade TW (1989) 'Skinfold thickness, body mass index and ischaemic heart disease', Epidemiol Community Health, 43, 3, 223-7.
- INDEC Instituto Nacional de Estadísticas y Censos (1997) 'La mortalidad en la Argentina entre 1980 y 1991', Serie Análisis Demográficos 9, Buenos Aires.
- Isner JM, Roberts WC, Heymsfield SB, Yager J (1985) 'Anorexia Nervosa and sudden death', Ann Int Med, 102, 49-52.
- Israelsson B (1988) 'Homocysteine and myocardial infarction', Atherosclerosis, 71, 227-34.
- Jenkins DJ, Wolever TM, Vuksan V, Brighenti F, Cunnane SC, Rao AV, Jenkins AL, Buckley G, Patten R, Singer W, et al (1989) 'Nibbling versus gorging: metabolic advantages of increased meal frequency', New Eng J Med, 321, 929-34.
- Johnson-Sabine E, Wood K, Patton G, Mann A, Wakeling A (1988) 'Abnormal eating attitude in London schoolgirls-a prospective epidemiological study: factors associated with abnormal response on screening questionnaires', Psych Med, 18, 3, 615-22.
- Kanis JA, Brown P, Fitzpatrick K, Hibbert DJ, Horn DB, Nairn IM, Shirling D, Strong JA, Walton HJ (1974) 'Anorexia Nervosa: a clinical, psychiatric and laboratory study', Quart J Med, 43, 170, 321-38.
- Kannel WB, Wolf PA, Castelli WP, D'Agostino RB (1987) 'Fibrinogen and risk of CVD: the Framingham study', J Am Med Assoc, 258, 1183-6.
- Kannel WB, D'Agostino RB, Belanger AJ, Sibershatz H, Tofler GT (1996) 'Long-term influence of fibrinogen initial and recurrent cardiovascular events in men and women', Am J Cardiology, 78, 1, 90-2.
- Knopp RH, Zhu X, Bonet B (1987) 'Effects of estrogens on lipoprotein metabolism and cardiovascular disease in women', Br J Hosp Med, 37, 4, 301-3.
- Kaplan RM, Wilson DK, Hartwell SL, Merino KL, Wallace JP (1985) 'Prospective evaluation of HDL cholesterol changes after diet and physical activity

conditioning programs for patients with type II diabetes mellitus', Diabetes Care, 8, 4, 343-8.

- Kaye WH, Gwirtsman HE, Obarzanek E, George T, Jimerson DC, Ebert MH (1986) 'Caloric intake necessary for weight maintenance in anorexia nervosa: nonbulimics require greater caloric intake than bulimics', Am J Clin Nutr, 44, 435-43.

- Kennedy SH, Brown GM, McVey G, Garfinkel PE (1991) 'Pineal and adrenal function before and after refeeding in anorexia nervosa', Biol Psych, 30, 216-24.

- Keys A et al (1950) 'The biology of human starvation', vol. II, University of Minnesota Press, Minneapolis, 968.

- Killen JD, Taylor CB, Hammer LD, Litt I, Wilson DM, Rich T, Hayward C, Simmonds B, Kraemer H, Varady A (1993) 'An attempt to modify unhealthy eating attitudes and weight regulation practices of young adolescent girls', Int J Eat Disord, 13, 369-84.

- King NA, Burley VJ, Blundell JE (1994) 'Exercise-induced suppression of appetite: effects on food intake and implications for energy balance', Eur J Clin Nutr, 48, 10, 715-24.

- Kiriike N, Iketan T, Nakanishi S, Nagata T, Inonue K, Okuno M, Ochi H, Kawakita Y.(1992) 'Reduced bone density and major hormnes regulating calcium metablism in anorexia nervosa', Acta Psych Scand, 86, 5, 358-63.

- Klag MJ, Ford DE, Mead LA, He J, Whelton PK, Liang KY, Levine DM (1993) 'Serum cholesterol in young men and subsequent cardiovascular disease', New Engl J Med, 328, 313-8.

- Klinefelter HF (1965) 'Hypercholesterolemia in anorexia nervosa', J Clin Endocr, 25, 1520-3.

- Klose et al (1976) 'Effect of fasting and effect of different diets on two post-heparin lipases', 4th Hungarian Atherosclerosis Conference.

- Knekt P, Reunanen A, Jarvinen R, Seppanen R, Heliovaara M, Aromaa A (1994) 'Antioxidant vitamin intake and coronary mortality in a longitudinal population study', Am J Epidemiol, 15, 139, 12, 1180-9.

- Kovanen PT, Brown MS, Goldstein JL (1979) 'Increased binding of low density lipoprotein to liver membranes from rats treated with 17 alpha ethinyl estradiol', J Biol Chemistry, 25, 254, 22, 11367-73.

- Kris-Etherton P and Yu S (1997) 'Individual fatty acid effects on plasma lipids and lipoproteins: human studies', Am J Clin Nutr, 65 Sup., 1628S-44.
- Kromhout D, Bosschieter EB, de Lezenne Coulander C (1982) 'Dietary fibre and 10-year mortality from coronary heart disease, cancer, and all causes. The Zutphen study', Lancet, 4, 3, 518-22.
- Laakso M (1996) 'Insulin resistance and coronary heart disease', Curr Opinion Lipid, 7, 217-26.
- LaCroix AZ, Mead LA, Liang KY, Thomas CB, Pearson TA (1986) 'Coffee consumption and the incidence of coronary heart disease', New Engl J Med, 315, 977-82.
- Landon J, Greenwood FC, Stamp TC, Wynn V (1966) 'The plasma sugar, free fatty acid, cortisol and growth hormone response to insulin and the comparison of this procedure with other tests of pituitary and adrenal function. II. In patients with hypothalamic and pituitary dysfunction of anorexia nervosa', J Clinical Investigation, 45, 4, 437-49.
- Langan S and Farrell P (1985) 'Vitamin E, vitamin A and essential fatty acid status of patients hospitalised for anorexia nervosa', Am J Clin Nutr, 41, 5, 1054-60.
- Lapidus L, Andersson H, Bengtsson C, Bosaeus I (1986) 'Dietary habits in relation to incidence of cardiovascular disease and death in women: a 12-year follow-up of participants in the population study of women in Gothenburg, Sweden', Am J Clin Nutr, 44, 444-8.
- Laségue C (1873) 'De l´anorexie hystérique', Archives Générales de Médicine, 1, 384-403.
- La Vecchia C, Franceschi S, Decarli A, Pampallona S, Tognoni G (1987) 'Risk factors for myocardial infarction in young women', Am J Epid, 125, 5, 832-43.
- Law MR, Frost CD, Wald NJ (1991) 'By how much does dietary salt reduction lower blood pressure? I: Analysis of observational data among populations', Brit Med J, 302, 811-9.
- Law M et al (1994) 'By how much and how quickly does reduction in serum cholesterol concentrations lower risk of ischemic heart disease?', Brit Med J, 308, 367-72
- Lejarraga H, Meletti I, Biocca S, Alonso V. (1986) 'Peso y talla de 15.214 adolescentes de todo el pais. Tendencia seculiar', Arch Argentino Pediatr, 84, 219.

- Lepage G and Roy C (1986) 'Direct transesterification of all classes of lipids in a one-step reaction', J Lipid Res, 27, 1, 114-20.
- Lentz S (1998) 'Mechanisms of thrombosis in hyperhomocystenemia', Curr Opin Hematol, 5, 5, 343-9.
- Lewis G, Ralevski E, Neitzert C, Uffelman K, Steiner G, Kennedy S (1994) 'Lipids and lipoproteins in anorexia nervosa before and after partial weight restoration', End Metabol, 1, 109-15.
- Lindholm L and Wilson GT (1988) ´Body image assessment in patients with bulimia nervosa and normal controls´, Int J Eat Disord, 7, 527-39.
- Luger A, Deuster PA, Kyle SB, Gallucci WT, Montgomery LC, Gold PW, Loriaux DL, Chrousos GP (1987) 'Acute hypothalamic-pituitary-adrenal responses to the stress of treadmill exercise. Physiologic adaptations to physical training', New Eng J Medicine, 21, 316, 21, 1309-15.
- Malozowski S, Muzzo S, Burrows R, Leiva L, Loriaux L, Chrousos G, Winterer J, Cassorla F (1990) 'The hypothalamic-pituitary-adrenal axis in infantile malnutrition', Clin Endocrinol (Oxf), 32, 4, 461-5.
- Manfredi R (2000) 'Management of dyslipidemia in patients with HIV disease', Clin Microbiol Infect, 6, 579-84
- Manson JE, Willett WC, Stampfer MJ, Colditz GA, Hunter DJ, Hankinson SE, Hennekens CH, Speizer FE (1995) 'Body weight and mortality among women', New Engl J Medicine, 333, 11, 677-85.
- Mant M and Faragher B (1972) 'The haematology of anorexia nervosa', Brit J Haematology, 23, 737-49.
- Marce L (1860) 'Note sur une forme de délire hypochondriaque consécutive aux dyspepsies et caratérisée principalment par le refus d´aliments', Annals Médico-Psychologiques, 6, 15-28.
- Marcos A, Varela P, Toro O, Lopez-Vidriero I, Nova E, Madruga D, Casas J, Morande G (1997) 'Interactions between nutrition and immunity in anorexia nervosa: a 1-year follow-up study', Am J Clin Nutr, 66, 2, 485S-90.
- Marsh A and Matheson J (1983) 'Smoking attitude and behaviour', HMSO, London.
- Martinez CA, Ibanez JO, Paterno CA, De Roig Bustamante MS, Itati Heitz M, Kriskovich Jure JO, De Bonis GR, Caceres LC (2001) 'Overweight and obesity in

children and adolescents of Corrientes city. Relationship with cardiovascular risk factor', Medicina (BA), 61, 3, 308-14.

- Martins Y, Pliner P, O'Connor R (1999) 'Restrained eating among vegetarians: does a vegetarian eating style mask concerns about weight?', Appetite, 32, 1, 145-54.

- Matzkin V, Grimble G, Robinson P (2001) 'Colesterol y homocisteína en anorexia nerviosa. Estudio preliminar', Bioquímica Patología Clínica, 65, 2/3, 28-33.

- Muldoon MF, Bachen AE, Manuck SB, Waldstein SR, Bricker PL, Bennett JA (1992) 'Acute cholesterol responses to mental stress and change in posture', Arch Int Medicine, 152, 4, 775-80.

- Mautner RD, Owen SV, Furnham A (2000) 'Cross-cultural explanations of body image disturbance in Western Cultural Samples', Int J Eat Disord, 28, 165-72.

- McAlister AL, Perry C, Maccoby N (1979) 'Adolescent smoking: onset and prevention', Pediatrics, 63, 650-57.

- McArthur L, Pena M, Holbert D (2001) 'Effects of socioeconomic status on the obesity knowledge of adolescents from six Latin American cities', Int J Obes Relat Met Disor, 25, 8, 1262-8.

- McCully K (1969) 'Vascular pathology of homocysteinemia: implications for the pathogenesis of arteriosclerosis', Am J Pathol, 56, 111-28.

- Mcgralth S and Gibney M (1994) 'The effects of altered frequency of eating on plasma lipids free-living healthy males on normal self-selected diets', Eur J Clin Nutr, 48, 402-7.

- McNamara JJ, Molot MA, Stremple JF, Cutting RT (1971) 'Coronary artery disease in combat casualties in Vietnam', J Am Med Assoc, 216, 1185-7.

- Meade TW, Mellows S, Brozovic M, Miller GJ, Chakrabarti RR, North WR, Haines AP, Stirling Y, Imeson JD, Thompson SG (1986) 'Haemostatic function and ischaemic heart disease: principal results of the Northwick Park heart Study', Lancet, 6, 533-7.

- Meade T et al (1993) 'Fibrinolytic activity, clotting factors, and long-term incidence of ischaemic heart disease in the Northwick Park Heart Study', Lancet, 30, 342, 8879, 1076-9.

- Meehan M and Katzman L (2001) 'Argentina the social body at risk', in Nassar M et al (eds.) 'Eating Disorders and Cultures in transition', Brunner Routledge, New York, 149-70.

- Mehler PS, Lezotte D, Eckel R (1998) 'Lipid levels in anorexia nervosa', Int J Eat Disord, 2, 217-21.
- Melton LJ, and Riggs BL. (1987) 'Epidemiology of age-related fractures', in Avioli LV (ed.) 'The Osteoporotic syndrome, detection, prevention and treatment'; Grune and Strattor, New York.
- Melton LJ, Chrischilles EA, Cooper C, Lane AW, Riggs BL (1992) 'Perspective. How many women have osteoporosis', J Bone Min Research, 7, 9, 1005-10.
- Mickley D (1999) 'Medical danger of bulimia nervosa and anorexia nervosa', in Lemberg R (ed.) 'Eating disorders. A reference sourcebook', Oryx Press, Phoenix, Arizona
- Miguez H (2001) 'Uso de tabaco en estudiantes de 13 a 15 años de la ciudad de Buenos Aires', Acta psiquiatrica psicologica de America Latina, 47, 2, 139-143.
- Mira M, Stewart PM, Abraham SF (1988) 'Managing hyperlipidemia', Medical Journal of Australia, 148, 316.
- Mira M, Stewart PM, Abraham SF (1989) 'Vitamin and trace element status of women with disordered eating', Brit Med J, 7, 303, 6815, 1474-5.
- Moesli P (1967) 'Anorexia Nervosa mit toedlichem Herzinfarkt', Schweiz Med Wschr, 97, 25-35.
- Moller-Madsen S and Nystrup J (1992) 'Incidence of Anorexia Nervosa in Denmark', Acta Psychiatr Scand, 85, 30-4.
- Moller-Madsen S, Nystrup J, Nielsen S (1996) 'Mortality in Anorexia Nervosa in Denmark during the period 1970-87', Acta Psychiatr Scand, 94, 454-59.
- Moniz C (1994) 'Alcohol and bone', Brit Med Bull, 50, 1, 67-75.
- Moodie D (1987) 'Anorexia and the heart. Results of studies to assess effects', Post Med, 81, 8,45-8.
- Mordasini R, Klose G, Greten H (1978) 'Secondary type II hyperlipoproteinemia in patients with anorexia nervosa', Metabolism, 27, 71-9.
- Moreira LB, Fuchs FD, Moraes RS, Bredemeier M, Duncan BB. Mosquera MT, Maurel DL, Pavon S, Arregui A, Moreno C, Vazquez J (1998) 'Alcohol intake and blood pressure: the importance of time elapsed since last drink', J Hypertension, 16, 2, 175-80.

- Moreiras-Varela O, Nunez C, Carbajal A, Morande G (1990) 'Nutritional status and food habits assessed by dietary intake and anthropometric parameters in anorexia nervosa. Int J Vitam Nutr Res, 60, 3, 267-74.
- Morgan J and Lacey J (1998) 'Salinophagia in anorexia nervosa', Brit J Psychiatry, 173, 352-3.
- Morgan H and Russell G (1975) 'Value of family background and clinical features as predictors of long-term outcome in anorexia nervosa: four-year follow-up study of 41 patients', Psychol Med, 5, 4, 355-71
- Morton R (1689) 'Phthisiologia seu exercitationes de phthisim, London, S. Smith.
- Mosquera M et al (1998) 'Incidence and risk factors in fractures of the proximal femur due to osteoporosis', Rev Panam Salud Publica, 3, 4, 211-9.
- Moss H, Bonicatto S, Korosci L, Girardelli AM, Murrelle L (1998) 'Substance abuse associated psychosocial problems among Argentina adolescents: sex heterogeneity and familial transmission', Drug Alcohol Dependency 1, 52, 3, 221-30.
- Moyano D, Vilaseca MA, Artuch R, Valls C, Lambruschini N (1998) 'Plasma total-homocysteine in anorexia nervosa', Eur J Clin Nutr, 52, 172-5.
- Moyano D, Sierra C, Brandi N, Artuch R, Mira A, Garcia-Tornel S, Vilaseca MA (1999) 'Antioxidant status in anorexia nervosa', Int J Eat Disord, 25, 99-103.
- Nakao M, Ando K, Nomura S, Kuboki T, Uehara Y, Toyooka T, Fujita T (2001) 'Depressive mood accompanies hypercholesterolemia in young Japanese adults', Jpn Heart J, 42, 6, 739-48.
- Nanchahal K, Ashton WD, Wood DA (2000) 'Alcohol consumption, metabolic cardiovascular risk factors and hypertension in women', Int J Epidemiol, 29, 1, 57-64.
- Nanjee M and Miller M (1989) 'Plasma lipoprotein and adrenocortical hormones in men, positive association of low density lipoprotein cholesterol with plasma cortisol concentration', Clin Chim Acta, 28, 180, 113-20.
- Nasser M (1986) 'Comparative study of the prevalence of abnormal eating attitudes among Arab female students of both London and Cairo universities', Psych Med, 16, 621-6.
- Nathan I, Hackett AF, Kirby S (1996) 'The dietary intake of a group of vegetarian children aged 7-11 years compared with matched omnivores', Brit J Nutr, 75, 533-44.

- National Cholesterol Education Program (NCEP): highlights of the report of the Expert Panel on Blood Cholesterol Levels in Children and Adolescents (1992), Pediatrics, 89, 3, 509-11.
- National Cholesterol Education Program (NCEP) Executive Summary of the Third Report. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) (2001), J Am Med Assoc, 285, 2486-97.
- National Task Force on the prevention and treatment of obesity. Weight cycling (1994), J Am Med Assoc, 19, 272, 15, 1196-202.
- Naurath HJ, Joosten E, Riezler R, Stabler SP, Allen RH, Lindenbaum J (1995) 'Effect of Vitamin B12, folate and vitamin B6 supplements in elderly people with normal serum vitamin concentrations', Lancet, 346, 85-9.
- Neaton JD and Wentworth D (1992) 'Serum cholesterol, blood pressure, cigarette smoking and death from coronary heart disease. Overall findings and differences by age for 316099 white men', Arch Int Med, 152, 56-64.
- Nelson L, Jennings GL, Esler MD, Korner PI (1986) 'Effect of changing levels of physical activity on blood pressure and haemodynamics in essential hypertension', Lancet, ii, 473-6.
- Nelson M (1993) 'Children's diet problems and solutions. Paper presented at National Dairy Council Conference', Children in Focus.
- Nelson M, Ash R, Mulvihill C, Peters T, Rogers P (2001) 'Longitudinal changes in iron status, cognitive function and physical activity in British adolescent girls', Final Report for DH/MRC Nutrition Research Programme, G9701928.
- Nestel PJ (1974) 'Cholesterol metabolism in anorexia nervosa and hypercholesterolemia', J Clin Endoc Met, 38, 2, 325-8.
- Neuman J, de Neumann MP, Valero E, Lindental D (1979) 'Epidemiology of coronary heart diseases risk factors in a free-living population', Preventive Medicine, 8, 445-62.
- Neville C (2002) 'A Pilot Study of Docosahexaenoic Acid in Anorexia Nervosa', Dietetic Research Project, King's College London.
- Neumark-Sztainer D, Story M, Resnick MD, Blum RW (1997) 'Adolescent vegetarians', Arch Pediat Adolescent Medicine, 151, 833-8.

- Neumark-Sztainer D, Story M, Dixon LB, Murray DM (1998a) 'Adolescents engaging in unhealthy weight control behaviours; are they at risk for other health-compromising behaviours?', Am J Public Health, 88, 6, 953- 5.
- Neumark-Sztainer D, Story M, Resnick MD, Blum RW (1998b) 'Lessons learned about adolescent nutrition from the Minnesota Health Survey', J Am Diet Assoc, 98, 12, 1449
- Neumark-Sztainer D, Story M, Falkner NH, Beuhring T, Resnick MD (1999) 'Sociodemographic and personal characteristics of adolescents engaged in weight loss and weight-muscle gain behaviours: who is doing what?', Preventive Medicine, 28, 40-50.
- NICE (2004) 'Eating disorders: Core interventions in the treatment and management of anorexia nervosa, bulimia nervosa and related eating disorders - NICE guideline', http://www.nice.org.uk (assessed 17/7/04)
- Newman M and Halmi K (1988) 'The endocrinology of Anorexia Nervosa and Bulimia Nervosa', Endocr Met Clin North America, 17, 195- 212.
- Newman MM and Halmi KA (1989) 'Relationship of bne density to estradiol and cortisol in anorexia nervosa and bulimia', Psych Res, 29, 1, 105-12.
- Norring CE and Sohlberg SS (1993) 'Outcome, recovery, relapse and mortality across six years in patients with clinical eating disorders', Acta Psych Scand, 87, 6, 437-44.
- Nova E, Varela P, Lopez-Vidriero I, Toro O, Cenal MJ, Casas J, Marcos A (2001) 'A one-year follow up study in anorexia nervosa. Dietary pattern and antropometrical evolution', Eur J Clin Nutr, 55, 7, 547-54.
- Nygard O, Nordrehaug JE, Refsum H, Ueland PM, Farstad M, Vollset SE (1997a) 'Plasma homocysteine levels and mortality in patients with coronary artery disease', New Eng J Medicine, 334, no. 4, 230-6.
- Obarzanek E, Lesem MD, Jimerson DC (1994) 'Resting metabolic rate of anorexia nervosa patients during weight gain', Am J Clin Nutr, 66, 666-75.
- O'Connor MA, Touyz SW, Dunn SM, Beumont PJ (1987) 'Vegetarianism in anorexia nervosa? A review of 116 consecutive cases', Med J Australia, 147, 540-2.
- Ogston D, Ogston WD (1976) 'The fibrinolytic enzyme system in anorexia nervosa', Acta Haematol, 55, 4, 230-3.

- Olson J (1996) 'Benefits and liabilities of vitamin A and carotenoids', J Nutrition, 126, 4 Sup.,1208S-12S.
- Pacin A, Martinez E, Martin de Portela ML, Neira MS (1999) 'Food consumption and intake of several nutrients in a population of the University of Luján, Argentina', Archivo Latinoamericano Nutrición, 49, 1, 31-9.
- Palmero HA and Caeiro A (1971) 'Epidemiology of hypertension in Cordoba, Argentina. I. Arterial pressure as a function of age and sex', Medicina (B Aires), 31, 5, 393- 403.
- PAN American Health Organisation-World Health Organisation (2002) Epidemiological Bulletin, 23, 3. http://www.paho.org (assessed 12th February 2003).
- Parthasarathy S (1987) 'Oxidation of LDL by thiol compounds leads to its recognition by the acetyl LDL receptor', Biochim Biophys Acta, 917, 337-40.
- Pasternack A (1970) 'Anorexia Nervosa, secondary aldosterism and angiopathy', Acta Medica Scandinavica, 187, 139-43.
- Patton GC, Johnson-Sabine E, Wood K, Mann AH, Wakeling A (1990) 'Abnormal eating attitude in London schoolgirls-a prospective epidemiological study: outcome at twelve month follow-up', Psych Med, 20, 2, 383-94.
- PDAY A Preliminary Report from the Pathobiological Determinants of Atherosclerosis in Youth (PDAY) research Group (1990) 'Relationship of atherosclerosis in young men to serum lipoprotein cholesterol concentrations and smoking', J Am Med Assoc, 264, 3018-24.
- Perkins KA, Epstein LH, Stiller RL, Marks BL, Jacob RG (1989) 'Acute effects of nicotine on resting metabolic rate in cigarette smokers', Am J Clin Nutr, 50, 3,545-50.
- Philipp E, Pirke K, Seidi M, Tuschi R, Fichter M, Eckert M, Wolfram G (1988) 'Vitamin Status in Patients with Anorexia Nervosa and Bulimia Nervosa', Int J Eat Disord, 8, 2, 209-18.
- Phinney SD, Tang AB, Waggoner CR, Tezanos-Pinto RG, Davis PA (1991) 'The transient hypercholesterolemia of major weight loss', Am J Clin Nutr, 53, 6, 1404-10.
- Pierce A and Guilpin E (1994) 'Historical analysis of tobacco marketing and the uptake of smoking by youth in the United States: 1890-1977. Special section Heath psychology and public health', Health Psychology, 14, 500-8

- Pirke KM, Fichter MM, Chlond C, Schweiger U, Laessle RG, Schwingenschloegel M, Hoehl C (1987) 'Disturbances of the menstrual cycle in bulimia nervosa', Clin Endocrinol, 27, 2, 245-51.
- Pollitt E and Mathews R (1998) 'Breakfast and cognition an integrative summary' Am J Clin Nutr, 67 Sup., 804-13.
- Pocock NA, Eismanj A, Hopper JL, Yeates MG, Sambrook PN, Eberl S. (1987) 'Genetic determinants of bone mass in adults. A twin study', J Clin Inv, 80,3, 706-10.
- Powers P and Fernandez R (1984) (eds.) 'Current treatment of anorexia nervosa and bulimia', Karger, New York.
- Powers P (1999) 'Osteoporosis and eating disorders', J Pediatr Adolesc Gynecol, 12, 2, 51.
- Pratt B and Woolfenden SR (2004) Interventions for preventing eating disorders in children and adolescents (Cochrane Review) in: The Cochrane Library, Issue 2, Chuchester, UK, John Willey and Sons, Ltd.
- Pugh E and Kates M (1984) 'The dietary regulation of acyltransferase and desaturase activities in nutritional membranes of rat liver', Lipids, 19, 48-55.
- Ratnasuriya RH, Eisler I, Szmukler GI, Russell GF (1991) 'Anorexia nervosa: Outcome and prognostic factors after 20 years', Brit J Psychiatry, 158, 495-502.
- Report of the Cardiovascular Review Group Committee on Medical Aspects of Food Policy (1995) 'Nutritional aspects of cardiovascular disease', 46.
- Refsum H and Nygard O (1998) 'Homocysteine and cardiovascular disease', Ann Rev Med, 49, 31-62.
- Riemersma RA, Wood DA, Macintyre CC, Elton RA, Gey KF, Oliver MF (1991) 'Risk of angina pectoris and plasma concentrations of vitamin A, C, and E and carotene', Lancet, 337, 1-5.
- RigottiNA, Nussbaum SR, Herzog DB, Neer RM. (1991) 'Osteoporosis in women with anorexia nervosa', N Engl J Med, 20, 311, 25, 1601-6.
- Robinson S, Skelton R, Barker M, Wilman C (1999) 'Assessing the diet of adolescent girls in the UK', Public Health Nutrition, 2, 4, 571-7.
- Robinson P (1993) 'Treatment for Eating Disorders in the United Kingdom. Part I. A survey of Specialist Services', Eat Disord Rev, 1, 1, 1-9.

- Robinson P and McHugh P (1995) 'A physiology of starvation that sustains eating disorders' in Szmukler G et al (eds.) 'Handbook of rating disorders: theory, treatment and research, J. Wiley, Chichester.
- Robboy MS, Sato AS, Schwabe AD (1974) 'The hypercarotenemia in anorexia nervosa: a comparison of vitamin A and carotene levels in various forms of menstrual dysfunction and cachexia', Am J Clin Nutr, 27, 4, 362-7.
- Rock CL, Gorenflo DW, Drewnowski A, Demitrack MA (1996) 'Nutritional characteristics, eating pathology and hormonal status in young women', Am J Clin Nutr, 64, 4, 566-71.
- Rock C and Curran-Celentano J (1994) 'Nutritional disorder of anorexia nervosa: a review', Int J Eat Disord, 15, 2, 187-203.
- Rock CL and Swendseid ME (1985) 'Plasma carotenoid levels in anorexia nervosa and in obese patients', Am J Clin Nutr, 42, 6, 1183-91.
- Rock C and Vasantharajan S (1995) 'Vitamin status of eating disorder patients: relationship to clinical indices and effect of treatment', Int J Eat Disord, 18, 3, 257-62.
- Rock C and Yager J (1987) 'Nutrition and Eating Disorders: A primer for clinicians', Int J Eat Disord, 6, 2, 267-80.
- Rosenvinge J and Mouland S (1990) 'Outcome and prognosis of anorexia nervosa. A restrospective study of 41 subjects', Brit J Psychiatry, 156, 92-7.
- Ross R (1999) 'Atherosclerosis-an inflammatory disease', N Engl J Med, 14, 340, 2, 115-26.
- Ruiz Rejon F, Martin-Pena G, Granado F, Ruiz-Galiana J, Blanco I, Olmedilla B (2002) 'Plasma status retinol, α and β tochopherols and main caroteins to first myocardial infarction: case control and follow-up study', Nutrition, 18, 26-31.
- Russell G (1967) 'The nutritional disorder in Anorexia Nervosa', J Psychosomatic Research, 11, 141.
- Russell G (1973) 'The Management of anorexia nervosa', in Robertson, R. and Proudfoot A (eds.) 'Symposium Anorexia Nervosa and Obesity', The Royal College of Physicians of Edinburgh.
- Russell J, Baur L, Beumont P, Byrnes S, Zipfel S (1998) 'Re-feeding of anorexics: wasteful not wilful', Lancet, 31, 352, 9138,1445-6.

- Sallis JF, Simons-Morton BG, Stone EJ, Corbin CB, Epstein LH, Faucette N, Iannotti RJ, Killen JD, Klesges RC, Petray CK, et al (1992) 'Determinants of physical activity and interventions in youth', Med Sci Sports Exersice, 24, 5248-57.
- Sample Size Calculator. http://www.ucalgary.ca (Assessed 10 January 2003).
- Sanchez-Muniz J, Cuesta C, Castro A (1990) 'Serum lipids and apolipoprotein B values, blood pressure and pulse rate in anorexia nervosa', Eur J Clin Nutr, 45, 33-6.
- Sanders T (1988) 'Essential and trans fatty acids in nutrition', Nutrition Research Review, 1, 56-78.
- Sanders T (1993) 'Marine oils: metabolic effects and role in human nutrition', Proceed Nutr Soc, 52, 457-72.
- Savendahl L and Underwood L (1999) 'Fasting increases serum total cholesterol, LDL cholesterol and apolipoprotein B in healthy, non-obese humans', J Nutrition, 129, 11, 2005-8.
- Schleimer K (1984) 'Dieting in teenage girls', Acta Paediatrica Scandinavica, Sup., 312.
- Schofield W et al (1985) 'Basal metabolic rate' Human Nutr: Clinical Nutr, 39C, Sup., 1, 1-96.
- Schorah CJ, Devitt H, Lucock M, Dowell AC (1998) 'The responsiveness of plasma concentration to small increases in dietary folic acid: a primary care study', Eur J Clin Nutr, 52, 407-11.
- Schreiber W, Schweiger U, Werner D, Brunner G, Tuschl RJ, Laessle RG, Krieg JC, Fichter MM, Pirke KM (1991) 'Circadian pattern of large neutral amino acids, glucose, insulin and food intake in anorexia nervosa and bulimia nervosa', Metabolism, 40, 5, 503-7.
- Schwartz D and Thompson M (1981) 'Do anorexics get well? Current research and future needs', Am J Psychiatry, 138, 3, 319-23.
- SEDRONAR Secretaría de Programación para la Prevención de la Drogadicción y la Lucha contra el narcotráfico (2001) 'Encuesta nacional de estudiantes de enseñanza media', Assessed 10 February, 2003, www.sedronar.gov.ar.
- Selles J, Coniglio R, Alvarez C, Betcher G and Perticarini L (1997) 'Detención precóz de niños y jóvenes con riesgo de enfermedad coronaria en edad adulta', Archivo Argentino Pediatría, 95, 76-83.

- Sharp C and Freeman C (1993) 'The medical complications of anorexia nervosa', Brit J Psychiatry, 162, 452-62.
- Shaper A (1988) 'Coronary heart disease: risk and reasons', Current Medical Literature Ltd., London.
- Sheffield (1984) 'Planning for prevention', Health Education J, 43, 57-59.
- Shekelle R and Stamper J (1984) 'Dietary cholesterol and ischaemic heart disease', The Lancet, I, 27, 1177-8.
- Shinder S and Sheohard R (1993) 'Relationship of premorbid mass and energy intake to increase of body mass during the treatment of anorexia nervosa', Int J Eat Disord', 14, 1, 65-73.
- Silverman J (1974) 'Anorexia Nervosa: Clinical observations in a successful treatment plan', J Pediatrics, 84, 68-73.
- Silverman J (1977) 'Clinical and metabolic observations in a successful treatment plan', in Vigersky R (ed.) 'Anorexia Nervosa a monograph of the National Institute of Child Health and Human Development', Raven, New York, 331-9.
- Silverman J (1983) 'Anorexia Nervosa. Clinical and metabolic observations', Int J Eat Disord, 2, 159-66.
- Simons-Morton BG, Taylor WC, Snider SA, Huang IW, Fulton JE (1994) 'Observed levels of elementary and middle school children's physical activity during physical education classes', Preventive Medicine, 23, 4, 437-41.
- Slone D, Shapiro S, Rosenberg L, Kaufman DW, Hartz SC, Rossi AC, Stolley PD, Miettinen OS (1978) 'Relation of smoking to myocardial infarction in young women', New Engl J Medicine, 8, 298, 1273-6.
- Smith SR, Bledsoe T, Chhetri MK (1975) 'Cortisol metabolism and the pituitary adrenal axis in adults with protein calorie malnutrition', J Clin End Metab, 40, 43-52.
- Smith A and Baghurst K (1992) 'Public health implications of dietary differences between social status and occupational category groups', J Epidemiol Comm Health, 46, 4, 409-16.
- Sosa Liprandi M, Gonzalez MA, Liprandi AS (1999) 'Tendencias de la mortalidad por infarto agudo de miocardio en la República Argentina durante el período 1980-1997', Rev Argentina de Cardiología, 67, 733-8.
- Sours J (1983) 'Case Reports of Anorexia Nervosa and Caffeinism', Am J Psychiatry, 140, 2, 235-6.

- Stampfer MJ, Hennekens CH, Manson JE, Colditz GA, Rosner B, Willett WC (1993) 'Vitamin E consumption and the risk of coronary diseases in women', New Engl J Medicine, 20, 328, 1444-9.
- Stampfer M and Colditz G (1991) 'Estrogens replacement therapy and coronary heart disease: a quantitative assessment of the epidemiological evidence', Preventive Medicine, 20, 47-63.
- Stec JJ, Silbershatz H, Tofler GH, Matheney TH, Sutherland P, Lipinska I, Massaro JM, Wilson PF, Muller JE, D'Agostino RB (2000) 'Association of fibrinogen with cardiovascular factors and cardiovascular disease in the Framingham offspring population', Circulation, 102, 1634-38.
- Stevenson DW, Darga LL, Spafford TR, Ahmad N, Lucas CP (1988) 'Variable effects of weight loss on serum lipids and lipoproteins in obese patients', Int J Obesity, 12, 6, 495-502.
- Steward DE, Raskin J, Garfinkel PE (1987) 'Anorexia nervosa, bulimia and pregnancy', Am J Obstet Gynecol, 157, 5, 1194-8.
- Stice E, Torst A and Chase A (2002) 'Healthy weight control and dissonance-based eating disorder prevention programs: results from a controlled trial' Int J Eat Disord, 33, 10-21.
- Stice E and Shaw H (2004) 'Eating disorder prevention programs; A meta-analytic review, Psych Bull, 130, 2, 206-27.
- Stone NJ (1994) 'Secondary causes of hyperlipidemia', Med Clin North America, 78, 1, 117-41.
- Stordy BJ, Marks V, Kalucy RS, Crisp AH (1977) 'Weight gain, thermic effect of glucose and resting metabolic rate during recovery from anorexia nervosa', Am J Clin Nutr, 30, 2, 138-46.
- Story M (1986) 'Nutrition management and dietary treatment of bulimia', Am Diet Assoc, 86, 4, 517-9.
- Striegel-Moore R, Dohm A, Pike, K, Wilfley D, Fairburn C (2002) 'Abuse, bullying and discrimination as risk factors for binge eating disorder', Am J Psych, 159, 11, 1902-7.
- Sullivan P (1995) 'Mortality in anorexia nervosa', Am J Psychiatry, 152, 7, 1073-75.

- Sullivan PF, Gendall KA, Bulik CM, Carter FA, Joyce PR (1998) 'Elevated Total Cholesterol in Bulimia Nervosa', Int J Eat Disord, 23, 425-32.
- Sutherland I and Willner P (1998) 'Pattern of alcohol, cigarette and illicit drug use in English adolescents', Addition, 93, 8, 1199-208.
- Stunkard AJ, Sorensen T, Schulsinger F (1983) 'Use of the Danish adoption register for the study of obesity and thinness' in Kety (ed.) 'The genetics of neurological and psychiatric disorders', Raven Press, New York.
- Szmulkler G (1985) 'The epidemiology of anorexia nervosa', J Psychiatric Research, 19, 2, 3, 143-153.
- Taboada Areso E (2000) 'Propuesta educativa para la prevención del abuso del alcohol en la educación secundaria obligatoria', Revista de Educación, 321, 317, 342.
- Tamai H, Mori K, Matsubayashi S, Kiyohara K, Nakagawa T, Okimura MC, Walter RM Jr, Kumagai LF, Nagataki S (1986) 'Hypothalamic-pituitary-thyroidal dysfunction in anorexia nervosa', Psychother Psychosom, 46, 3, 127-31.
- Task Force Report (1998) 'Prevention of coronary heart disease in clinical practice: Recommendations of the Second Joint Task Force of European and other Societies on Conorary Prevention', Atherosclerosis, 140, 199-270.
- Tauchmanova L, Rossi R, Biondi B, Pulcrano M, Nuzzo V, Palmieri EA, Fazio S, Lombardi G (2002) 'Patients with subclinical cushing's syndrome due to adrenal adenoma have increased cardiovascular risk', Clin End Metab, 87, 487-8.
- Thibault L and Roberge A (1987) 'The Nutritional Status of Subjects with Anorexia Nervosa', Int J Vit Nutritional Research, 57, 447-52.
- Thompson J and Altabe M (1991) 'Psychometric qualities of the Figure Rating Scale, Int J Eat Disord, 10, 615-9.
- Thompson RA and Sherman R (1993) 'Helping Athletes with Eating Disorders', Human Kinetics Publishers, USA.
- Lindholm L and Wilson GT (1988) 'Body image assessment in patients with bulimia nervosa and normal controls', Int J Eat Disord, 7, 527-39.
- Theander S (1985) 'Outcome and prognosis of anorexia nervosa and bulimia: some results of previous investigations, compared with those of a Swedish long-term study', J Psychiatry Research, 19, 493-508.

- Thurnham D, Smith E, Flora PS (1985) 'Concurrent Liquid Chromatography assay of retinol, α tocohherol, β carotene, α carotene, lycopene, and β cryptoxantin in plasma with tocopherol acetate as internal standard', Clin Chem, 32, 2, 377-81.
- Tietyen J and Fleming K (1995) 'Nutrient intake of breakfast vs. non breakfast eaters', J Am Diet Assoc, 95, Sup. A, 55.
- Tikkanen MJ, Nikkila EA, Vartiainen E (1978) 'Natural oestrogen as an effective treatment for type II hyperlipoproteinemia in post menopausal women', Lancet 2, 40-2.
- Touyz S and Beumont P (ed.) (1985) 'Eating disorders. Prevalence and treatment', Williams and Wilkins Adis. Pty. Ltd., Sydney.
- Treasure J, Wheeler MJ, Safieh B, Russell GF (1985) 'Anorexia nervosa and the adrenal: the effect of weight gain', J Psychiatry Research, 19, 2-3, 221-5.
- Treasure J and Russell G (1988) 'Intrauterine growth and neonatal weight gain in babies of women with Anorexia Nervosa', Brit Med J, 296, 1038.
- Treasure JL, Russell GF, Fogelman I, Murby B. (1987) 'Reversible bone loss anorexia nervosa', Br Med J, 22, 295, 474-5.
- Troter K (1997) 'Nutrition and eating disorders', Nurs Times, 12-18, 46, Sup. 1-6.
- Turnbull S, Ward A, Treasure J, Jick H, Derby L (1996) 'The demand for eating disorder care: an epidemiological study using the general practice research database', Brit J Psychiatry, 169, 705-12.
- Turner JG, Gilchrist NL, Ayling EM, Hassall AJ, Hooke EA, Sadler WA (1992) 'Factors affecting bone mineral density in high school girls', New Zealand Med J, 25, 105, 930, 95-6.
- Young MH, Jeng CY, Sheu WH, Shieh SM, Fuh MM, Chen YD, Reaven GM.
- Ubbink JB, Vermaak WJ, van der Merwe A, Becker PJ (1993) 'Vitamin B-12, vitamin B-6 and folate nutritional status in men with hyperhomocysteinemia', Am J Clin Nutr, 57, 47-53.
- Ueland M and Refsum H (1989) 'Plasma homocysteine a risk factor for vascular disease: plasma levels in health, disease and drug therapy', J Lab Clin Med, 5, 114, 473-501.
- Ueland PM, Refsum H, Beresford SA, Vollset SE (2000) 'The controversy over homocysteine and cardiovascular risk', Am J Clin Nutr, 72, 324-32.

- Uhe AM, Szmukler GI, Collier GR, Hansky J, O'Dea K, Young GP (1992) 'Potential regulators of feeding behaviour in anorexia nervosa', Am J Clin Nutr, 55, 1, 28-32.
- Umeki S (1988) 'Biochemical Abnormalities of Serum in Anorexia Nervosa', J Nervous Mental Disease, 176, 8, 503-6.
- Vaisman N, Tabachnik E, Hahn T, Voet H, Guy N (1992a) 'Superoxide production during refeeding in patients with anorexia nervosa', Metabolism, 41, 1097-9.
- Vaisman N, Wolfhart D, Sklan D (1992b) 'Vitamin A metabolism in plasma of normal and anorexic women', Eur J Clin Nutr, 46, 12, 873-8.
- Van Binsbergen CJ, Hulshof KF, Wedel M, Odink J, Coelingh Bennink HJ (1988a) 'Food preferences and aversions and dietary pattern in anorexia nervosa patients', Eur J Clin Nutr, 42, 671-8.
- Van Binsbergen CJ, Odink J, Van den Berg H, Koppeschaar H, Coelingh Bennink HJ (1988b) 'Nutritional Status in Anorexia Nervosa: Clinical Chemistry, Vitamins and Iron', Eur J Clin Nutr, 42, 11, 929-37.
- Vandereyck W and Pierloot R (1983) 'Drop-out during inpatient treatment of anorexia nervosa', A clinical study of 133 patients', Brit J Med Psychology.
- Van Deth R and Vandereyecken W (1988) 'Miraculous Fast to Morbid Pursuit of Thinness: Anorexia nervosa in Historical Perspective', Amsterdam, Boompers.
- Vega GL, Groszek E, Wolf R, Grundy SM (1982) 'Influence of polyunsaturated fats on composition of plasma lipoproteins and apolipoproteins', J Lipid Res, 23, 6, 811-22.
- Verschuur M, Bekkers M, Van Erck M, Emeis J, De Matt M (2000) 'Influence of plasma triglyceride and plasma cholesterol levels on the clearance rate of fibrinogen', Ann N Y Acad Scic, 936, 639-42.
- Vlassara H, Spiegel RJ, San Doval D, Cerami A (1986) 'Reduced plasma lipoprotein lipase activity in patients with malignancy-associated weight loss', Horm Metab Res, 18, 10, 698-703.
- Wachslicht-Rodbard H, Gross HA, Rodbard D, Ebert MH, Roth J (1979) 'Increased insulin binding to erythrocytes in anorexia nervosa: restoration to normal with refeeding', New Engl J Med, 19, 300, 16, 882-7.

- Wade DP, Knight BL, Soutar AK (1988) 'Hormonal regulation of low-density lipoprotein (LDL) receptor activity in human hepatoma Hep G2 cells. Insulin increases LDL receptor activity and diminishes its suppression by exogenous LDL', Eur J Biochem, 16, 174, 1, 213-8.
- Wald DS, Law M, Morris JK (2002) 'Homocysteine and cardiovascular disease: evidence on causality from a meta-analysis', BMJ, 325, 23, 1202-7.
- Wardle J and Marsland L (1999) 'Adolescent concerns about weight and eating; a social-development perspective', J Psychosomatic Research, 34, 4, 337-91.
- Warren M and Vande Wille R (1973) 'Clinical and metabolic features of anorexia nervosa', Am J Obstet Gynecol, 117, 435-49.
- Wei M, Gibbons LW, Mitchell TL, Kampert JB, Stern MP, Blair SN (2000) 'Low fasting plasma glucose level as a predictor of cardiovascular disease and all-cause mortality', Circulation, 101, 2047-52.
- Whitaker A, Johnson J, Shaffer D, Rapoport JL, Kalikow K, Walsh BT, Davies M, Braiman S, Dolinsky A (1990) 'Uncommon troubles in young people: prevalence estimates of selected psychiatric disorders in a non-referred adolescent population', Arch Gen Psychiatry, 47, 5, 487-96.
- WHO (1990) 'Diet, nutrition and prevention of chronic diseases', Report of the WHO study group, Geneva.
- WHO (1994) 'Assessment of fracture risk and its application to screnning for postmenopausal osteoporosis', 843 Technical Report Series, Geneva.
- Wiederman M and Pryor T (1996) 'Substance use and impulsive behaviours among adolescents with eating disorders', Addict Behav, 21,2, 269-72.
- Wilhelmsen L, Svardsudd K, Korsan-Bengtsen K, Larsson B, Welin L, Tibblin G (1984) 'Fibrinogen as a risk factor for stroke and myocardial infarction', New Engl J Medicine, 311, 501-5.
- Wilson JH, Rietveld T, Van den Berg JW, Jansen H, Swart GR, Lamberts SW (1989) 'The effect of very low energy diets on the fatty acid composition of serum lipids', Int J Obesity, 13 Sup. 2, 51-60.
- Williams C (1997) 'Cardiovascular risk factors in Women', Proced Nutr Soc, 56, 1B, 383-91.

- Windauer U, Lennerts W, Talbot P, Touyz SW, Beumont PJ (1993) 'How well are 'cured' anorexia nervosa patients? An investigation of 16 weight-recovered anorexic patients', Brit J Psychiatry, 163, 195-200.
- Willet W (1998) 'Nutrition Epidemiology', 2nd, Oxford University Press, Oxford.
- Witteman JC, Grobbee DE, Kok FJ, Hofman A, Valkenburg HA (1989) 'Increased risk of atherosclerosis in women after menopause', Brit Med J, 11, 298, 6674, 642-4.
- Wood D, De Backer G, Faergeman O, Graham I, Mancia G, Pyorala K (1998) 'Prevention of coronary heart disease in clinical practice: Recommendations of the Second Joint Task Force of European and other Societies on Coronary Prevention', Atherosclerosis, 140, 199-270.
- Worsley A and Skrzypier G (1998) 'Teenage vegetarianism: prevalence, social and cognitive contexts', Appetite, 30, 2, 151-70
- Young CM, Ringler I, Greer BJ (1953) 'Reducing and post-reducing maintenance on the moderate-fat diet', J Am Diet Assoc, 29, 890-6
- Zimmet P, Alberti KG, Shaw J (2001) 'Global and societal implications of the diabetes epidemic, Nature, 414, 12, 782-7.
- Zock PL, Katan MB, Merkus MP, van Dusseldorp M, Harryvan JL (1990) 'Effect of a lipid rich fraction from boiled coffee on serum cholesterol', Lancet, 335, 1235-7.
- Zuckerfeld R, Fuchs A, Cormillot A (1998a) 'Caracterización y detención de bulimia en la ciudad de Buenos Aires', Acta Psiquiatrica Psicológica America Latina, 34, 298-302.
- Zuckerfeld A, Zuckerfeld R, Quiroga S (1998b) 'Conducta alimentaria, peso corporal y psicopatología en mujeres jovenes', Revista Argentina de Clínica Pscicológica, VII, 1-19.
- Zumoff B, Walsh BT, Katz JL, Levin J, Rosenfeld RS, Kream J, Weiner H (1983) 'Subnormal plasma dehydroisoandrostrerone to cortisol ratio in anorexia nervosa: a second hormonal parameter of ontogenetic regression', J Clin Endocr Metab, 56, 668-72.
- Zuniga-Guajardo S, Garfinkel PE, Zinman B (1986) 'Changes in insulin sensitivity and clearance in anorexia nervosa', Metabolism, 35, 1, 1096-100.

- Zwaan M and Mitchell J (1993) 'Medical complications in anorexia and bulimia nervosa', in Kaplan A and Garfinkel P (eds.) 'Medical Issues and the Eating Disorders', Brunnel-Mazel, New York, 60-100.

Appendices

Appendix 1.1. Cholesterol biosynthesis and regulation

Cholesterol biosynthesis and regulation

Cholesterol is a sterol, having the skeleton of the cyclopentanoperhydrophenanthrene. It is a structural component of the plasma membranes of cells, a precursor of the synthesis of steroid and adrenal hormones, and of Vitamin D. Cholesterol is synthesised in the liver from acetate (Figure 1.2). Each day the liver synthesises an amount of new bile acid from cholesterol equal to the amount of acidic sterols lost in the faeces. When the pool of cholesterol is high, de novo synthesis is regulated by the following mechanisms:

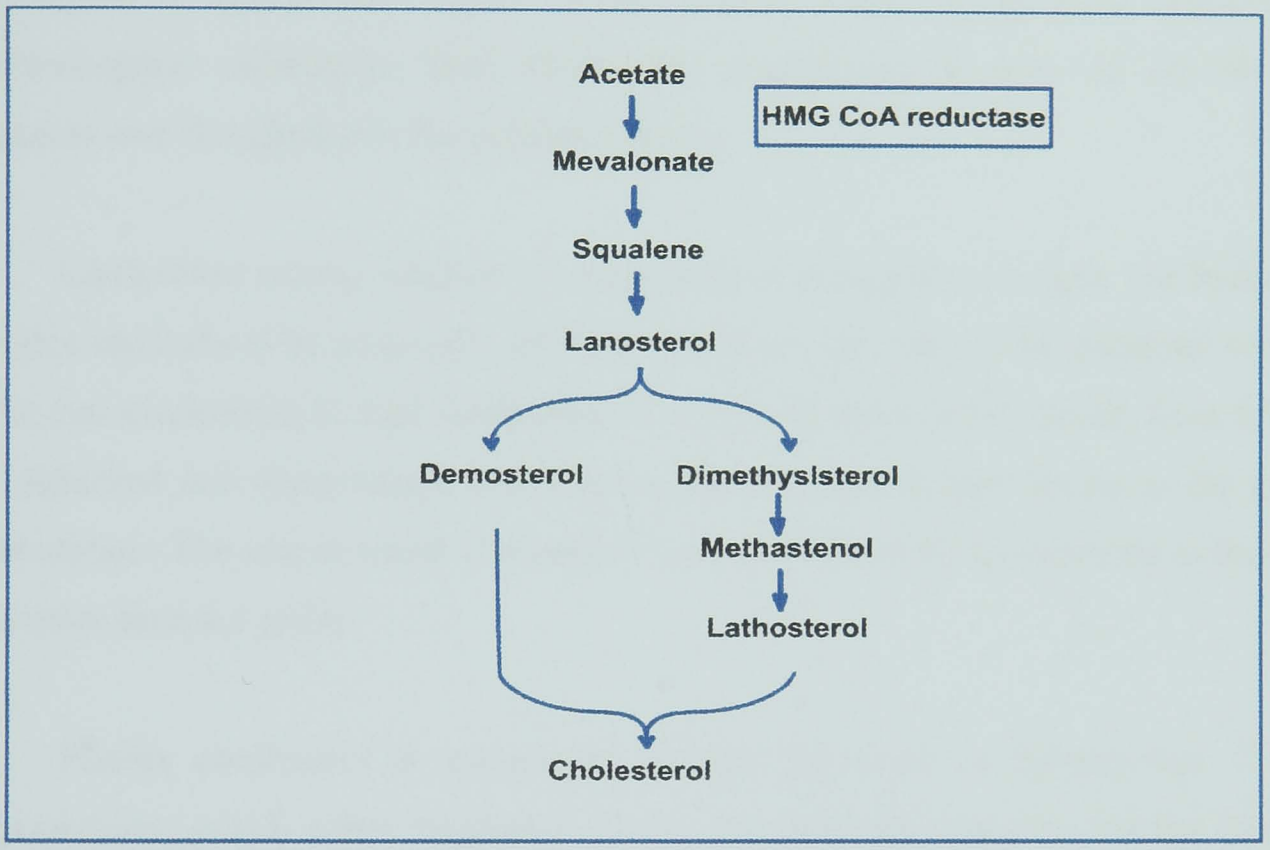
1. Inhibition of HMG CoA reductase (hydroxymethylglutaryl CoA reductase), key enzyme in the synthesis of cholesterol which catalyses the conversion of acetate into mevalonate
2. Inhibition of LDL receptor synthesis when cholesterol enters the cells
3. Stimulation of cholesterol 7α hydroxylase, key enzyme in the conversion of cholesterol to bile acids, which promotes cholesterol excretion.
4. Stimulation of cholesterol acyltransferase (ACAT), key enzyme which esterifies free cholesterol promoting cholesterol storage in the cells.

The LDL receptor co-ordinates intracellular and extracellular concentrations of cholesterol, maintaining a constant level of cellular cholesterol in face of possible changing blood lipoproteins. Intracellular levels of cholesterol, hormones and the rate-limiting enzymes of cholesterol synthesis (HMGCoA reductase) and degradation (7α hydroxylase) regulate LDL receptor synthesis. Subjects with familial hyperlididemia type II have elevated total cholesterol and LDL levels, caused by a reduced number of LDL receptors, which leads to the deposition of atheroclerotic plaque in the arteries.

Appendix 1.1. Cholesterol biosynthesis and regulation continue…

Figure A1.1: Pathway of cholesterol synthesis

Source: Farkkila et al (1988)



Cholesterol absorption and metabolism

Cholesterol reaches the intestinal lumen from two sources: 1) exogenous or dietary cholesterol, and 2) endogenous cholesterol. Exogenous cholesterol is derived from the ingestion of animal foods (meat, eggs, whole fat dairy products, etc.). Endogenous cholesterol comes from biliary acids re-absorbed through the enterohepatic circulation, from cholesterol synthesised de novo in the intestinal mucosa and sloughed into the intestinal lumen.

Cholesterol esters, whether of exogenous or endogenous origin, are hydrolysed to free cholesterol by pancreatic cholesterol esterase present in the intestinal contents. The free cholesterol is then solubilised in mixed micelles of bile acids, from which it is absorbed and incorporated into Chylomicrons (CM) to gain access to the general circulation. The rate at which cholesterol can be absorbed by the intestine is limited to no more than 0.5 g/day.

Plasma cholesterol is transported through the body as lipoproteins. CM are lipoproteins, which carry exogenous dietary fat, from the intestine via the lymph to the blood and the liver (Table A1.2). CM gains Apolipoprotein (apo) A-I, A-II, A-IV and B48 in its development, and additional apolipoproteins, apo C-II and apo E, are transferred from HDL. This is so that CM can interact with the enzyme lipoprotein lipase (LPL), located in the surface of the adipose and skeletal muscle tissues. Apo C-II is required as a cofactor of the activation of LPL, which hydrolyses CM triglycerides into unesterified fatty acid and glycerol. The cholesterol rich CM remnants are taken up by a specific hepatic receptor that recognises apo E (Figure A1.2).

Appendix 1.2. Cholesterol absorption and metabolism continue...

The liver secretes into the circulation, in a very low-density lipoprotein (VLDL). VLDL is hydrolysed by LPL and the result is the production of VLDL remnant, which is taken by the liver, and intermediate density lipoproteins (IDL). IDL contains 40% of triglycerides, 30% of cholesterol and 20% of phospholipids. Later, IDL is taken up by an hepatic receptor, but the remaining half is converted to LDL with apoB-100 as a remnant peptide. LDL and IDL are the major sources of cholesterol that accumulate in atherosclerotic plaques. LDL receptors at the surface of the cells are important in the uptake of this lipoprotein and in regulating the cholesterol content of cells and plasma.

The liver secretes HDL. Through the action of the plasma enzyme lecithin-cholesterol acyl transferase (LCAT), acyl groups from phosphatidylcholine are transferred from the free cholesterol from the tissues, other lipoproteins and/or HDL itself to produce cholesterol esters, which accumulate in the central core of the HDL particles. This process permits the transport of cholesterol from peripheral tissues to lipoproteins, resulting in its the removal via the liver.

LDL delivers cholesterol to the tissues. In contrast HDL promotes the removal of the excess cholesterol from cells. High concentrations of LDL and low concentrations of HDL have been highly associated with greater risk of cardiovascular disease (Cholesterol Education Program 2002).

Appendix 1.2. Cholesterol absorption and metabolism continue...

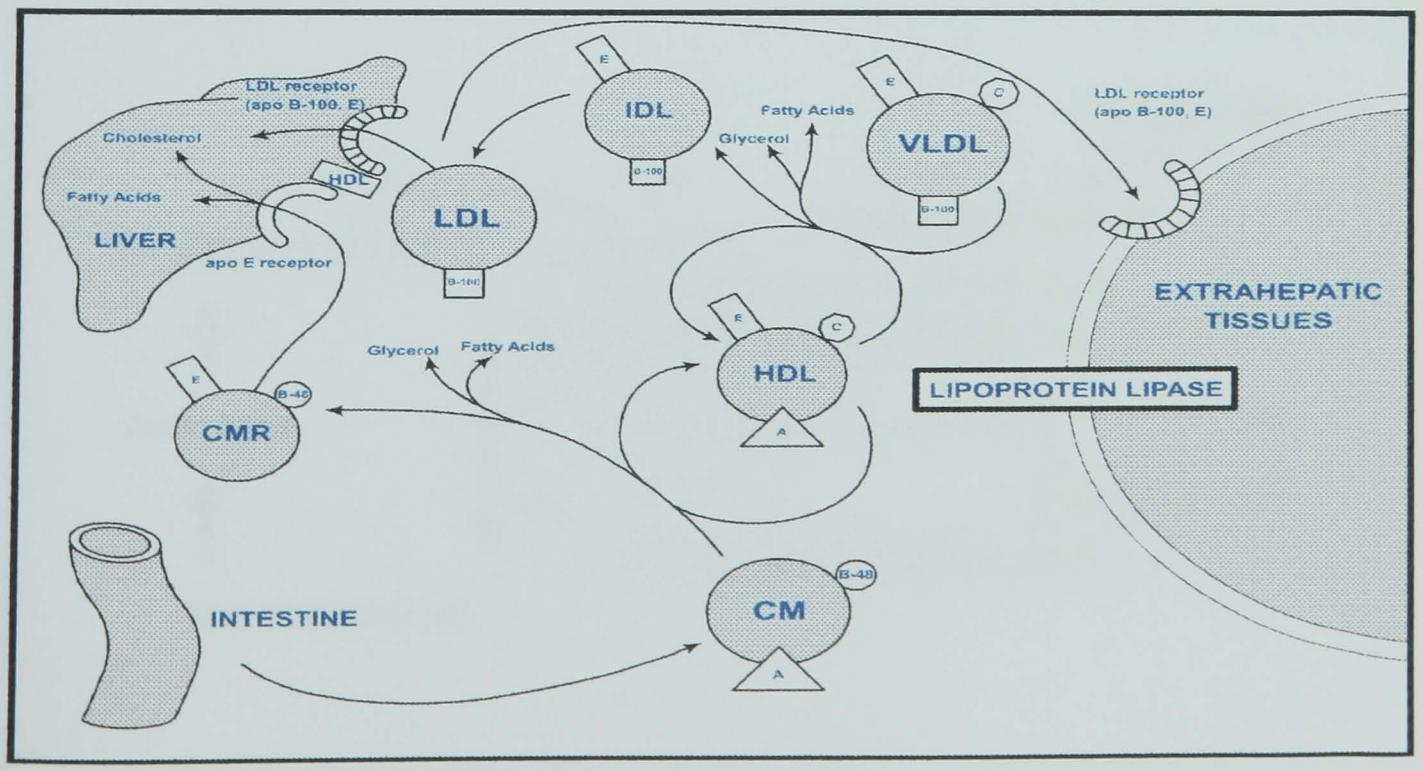
Table A1.2: The average composition of blood lipoproteins and their functions.

Source: Gunstone (1992).

Lipoprot	Apoproteins	Composition (% total) TG	Cholesterol	Phospho lipid	Protein	Metabolism	Simplified role
CM	Apo AI Apo B48 Apo C Apo AII Apo E	90	5	3	2	Catabolism by extrahepatic lipoprotein lipase to remmants with hepatic fate	Transport of dietary TG and cholesterol
VLDL	Apo B 100 Apo CI Apo CII Apo CIII Apo E	60	12	18	10	Catabolism by extrahepatic LPL to LDL or remnant	Transport of endogenously synthesised TG
LDL	Apo AI Apo AII Apo B100 Apo C	10	50	15	25	Product of VLDL catabolism	Transport of cholesterol to peripheral tissues
HDL	Apo AI Apo AII Apo CI Apo CII Apo CIII Apo D Apo E	5	20	25	50	Precursor secreted by liver, plasma particle derived from the action of LPL and LCAT	Donor/Acceptor of protein to/from other lipoproteins. Transport of peripheral tissues cholesterol to the liver

Figure A1.2: Cholesterol transport and metabolism

Source: Harper (2000)

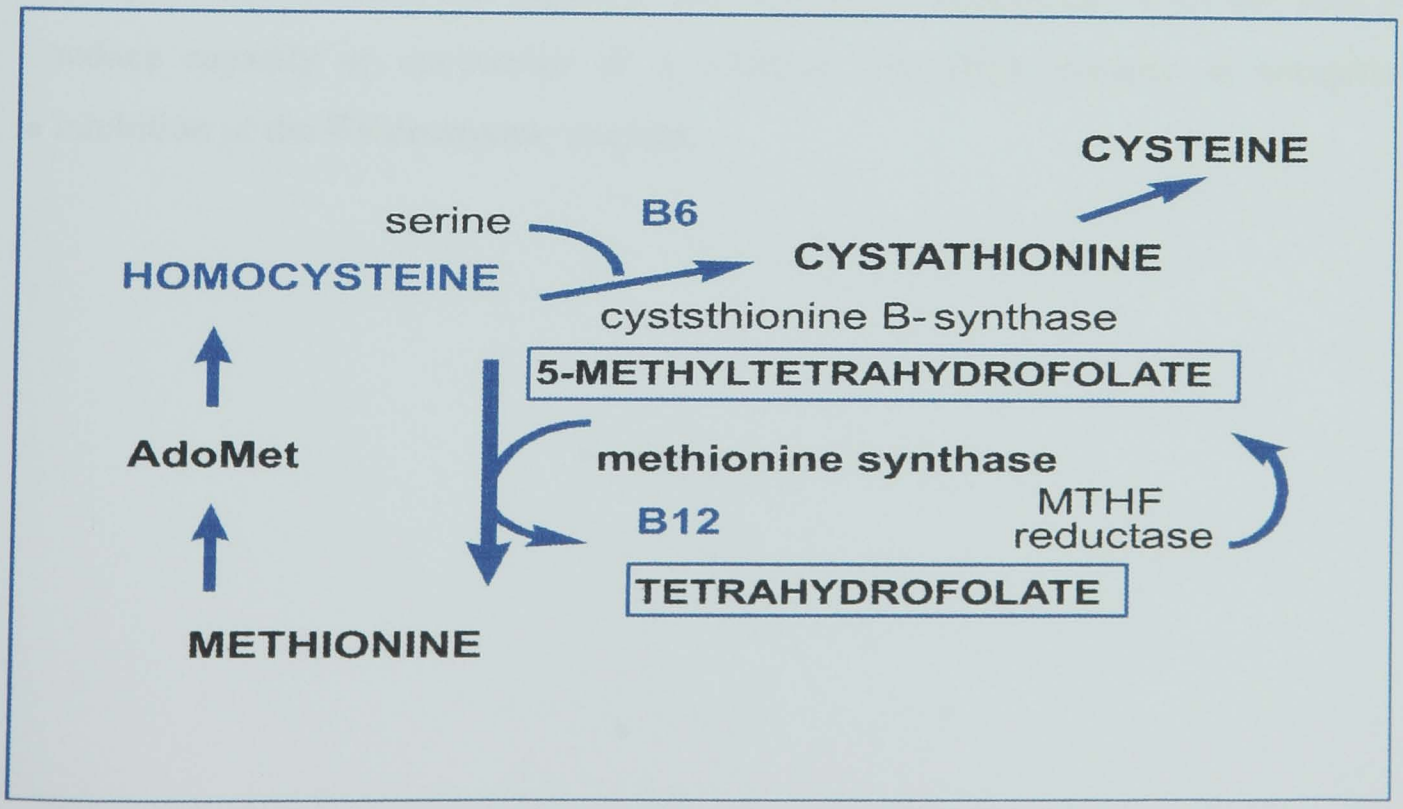


Appendix 1.3. Homocysteine metabolism

Intracellular homocysteine is either remethylated to methionine or is condensed with serine to form cystathionine. The cystathione formation is catalysed by the enzyme cystathionine B- synthase. This enzyme requires pyridoxal 5' phosphate, the biological active form of vitamin B6, as a cofactor. The conversion of homocysteine to methionine is either catalysed by betaine-homocysteine methyltransferase or methyltetrahydrofolate-homocysteine methyltransferase (methionine synthase). Methionine synthase requires 5-methyltetrahydrofolate (5-MTHF), which provides a methyl group, and vitamin B12 is a cofactor. 5-MTHF is converted to tetrahydrofolate (THF) in this reaction. The formation of methionine occurs principally in the liver. S-Adnosylmethionine (AdoMet) is an activator of cystathionine B-synthase, the enzyme that catalyses the transsulfuration reaction, and an inhibitor of MTHF reductase: the enzyme that generates THF. In Folate and vitamin B12 deficiency there is a depletion of AdoMet, and therefore an inhibition in the transsulfuration pathway. Consequently, homocysteine is accumulated, because it cannot be metabolised by either of the two pathways (Figure 1.5).

Figure A1.3: Homocysteine metabolism

Source: Ueland and Refsum (1989)



Appendix 1.4. Roles and metabolism of fatty acids

EFA have major roles in the organism:

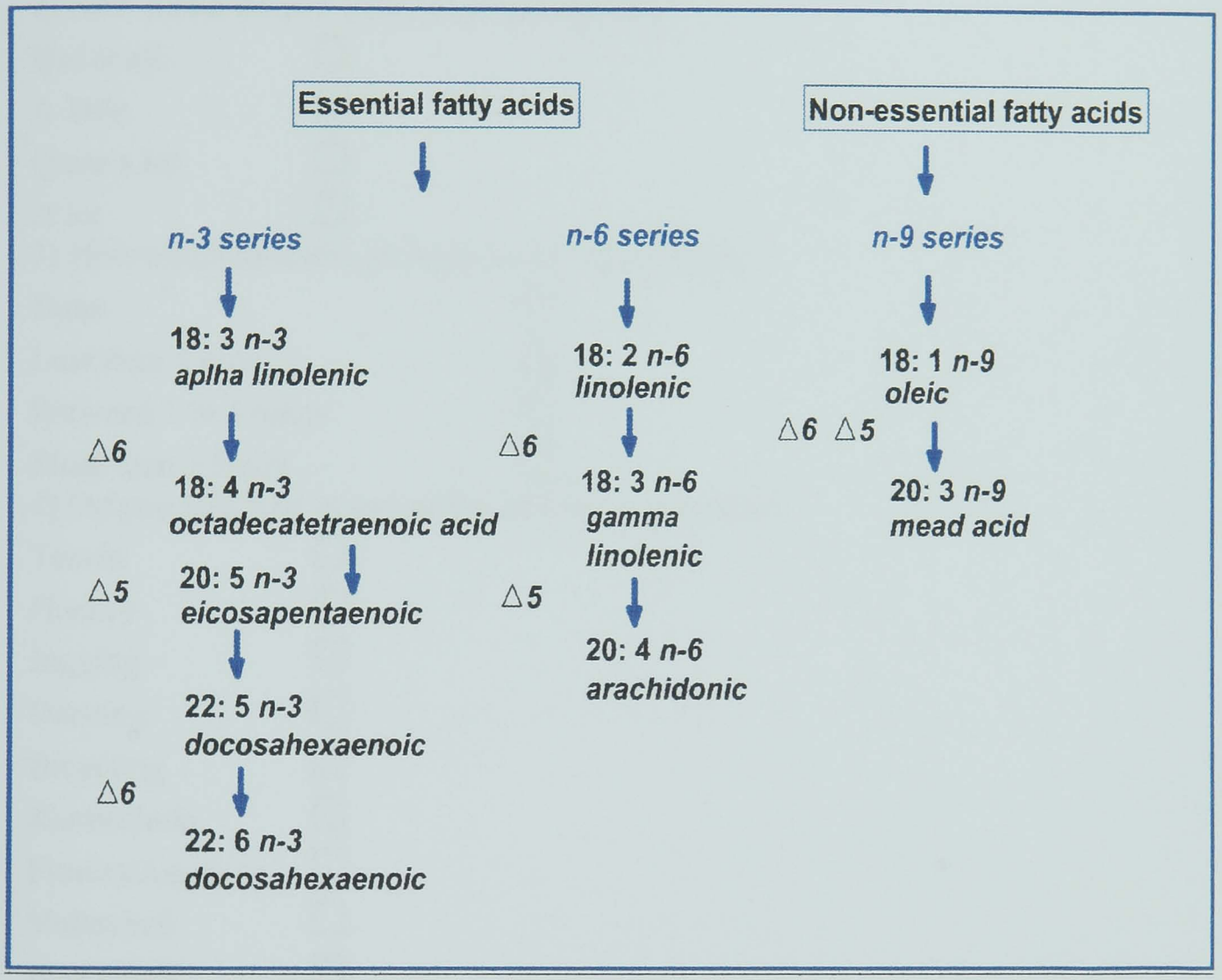
1. They are required for structural integrity of all cells of an organism. Because of their unsaturation they offer properties of fluidity and permeability to membranes. They can also change the binding ability of substances to their receptors. For example, they may regulate steroid receptors.
2. They are precursors for eicosanoids, prostaglandins and leukotrienes.
3. They are required for the maintenance of the impermeability of the skin. In the absence of adequate levels of essential fatty acids, water is lost through the skin, producing thirst and small volumes of concentrated urine.
4. *N*-6 derivatives are effective cholesterol lowering agents and the elevation of cholesterol, in turn, inhibits ↖6 and ↖5 desaturation, decreasing the formation of derived EFA and this inhibits further elevation of cholesterol.
5. *N*-3 derivatives are effective in decreasing VLDL and triglyceride values.

Both linoleic acid and linolenic acid compete for the same elongase and desatuarase enzymes. The same enzyme system handles the fatty acids of the similar chain lengths. This means that if there are large amounts of non-EFA, they will compete with the EFA and suppress their activity. A high intake of linoleic acid may reduce capacity or conversion of α linolenic into DHA because of competitive inhibition of the ↖6 desaturase enzyme.

Appendix 1.4. Roles and metabolism of fatty acids **continue...**

Figure A1.4: Metabolism of polyunsaturated fatty acids

Source: Sanders (1988)



Nomenclature note: example 18: 2 *n*-6, ***18*** refers to the number of carbons, ***2*** represents the number of double bonds and ***n-6*** or ***w-6*** means that the double bond is six carbon atoms from the terminal methyl group. Δ5 and Δ6 are desaturase enzymes, which add double bonds in the carbons 5 and 6, respectively, from the terminal acidic group.

Appendix 2.1. Life-style questionnaire and background data

This questionnaire is Confidential and any publication of this data will not include any details which can be used to identify you. Please tick answer if appropriate.

Date: _____

1) Rate on the following scale of 1 to 10 (where '1' is not at all healthy and '10' is the very healthy), how healthy you think you are. Ring the appropriate number.

1 2 3 4 5 6 7 8 9 10

2) How much do you enjoy physical activity?

Not at all ☐
A little ☐
Quite a lot ☐
A lot ☐

3) How much time do you spend walking each day?

None ☐
Less than 1 hour ☐
Between 1 to 3 hours ☐
More than 3 hours ☐

4) Do you take part in any of the following activities?

Tennis ☐
Hockey ☐
Jogging ☐
Dancing ☐
Bicycling ☐
Karate/Judo ☐
Fitness/Aerobics ☐
Volleyball ☐
Basketball ☐
Swimming ☐
Other ☐ Which?_____

5) How many times in the last 7 days have you taken any physical activity for long enough to breath harder?

Never ☐ *go to question 7*
Once ☐
Twice ☐
3 times or more ☐

6) For how long each time?

Less than half an hour ☐
Between half and one hour ☐
More than one hour ☐

Appendix 2.1. Life-style questionnaire and background data continue...

7) Do you smoke tobacco?

YES ☐

NO ☐

8) If yes, how many cigarettes?

Less than 5/day? ☐

5 to 10/day? ☐

11 to 15/day? ☐

16 or more /day? ☐

9) Which statement describes you best?

- I have never smoked at all, not even a puff ☐
- I have tried smoking once or twice ☐
- I used to smoke, but I don't now ☐
- I smoke occasionally, less than one cigarette a week ☐
- I smoke regularly, but would like to give up ☐
- I smoke regularly and I don't want to give up ☐

10) How many people in your home (including yourself) smoke?

0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ more ☐

11) Do any of these people smoke on most days?

Mother ☐

Father ☐

Brother ☐

Sister ☐

Close friend ☐

12) During the last 7 days on how many days did you drink alcohol?

0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 or more ☐ *If 0 was chosen go to question 15*

13) During the last 7 days how much of the following alcoholic drinks have you drunk?

I drank _____ *glass/es of wine*

_____ glass/es of beer

_____ glass/es of cider

_____ glass/es of Martini, Cinzano

_____ glass/es of spirit (whisky, vodka, etc.)

_____ of something else. Please write

Appendix 2.1. Life-style questionnaire and background data **continue...**

14) Have you drunk alcoholic drinks at any of these places during the last 7 days?

I drank alcohol at home ☐
I drank alcohol at a friend's ☐
I drank alcohol at a disco/party ☐
I drank alcohol at a pub/bar ☐
I drank alcohol outside a public place (street, park, etc) ☐
Other ☐ Specify_____

15) Do you usually drink coffee?

YES ☐
NO ☐ *go to question 18*

16) If yes, how much per day? 1 cup = 200 ml

Less than 1 cup ☐
1 cup ☐
2 cups ☐
3 cups ☐
4 or more cups ☐

17) What type of coffee do you usually drink?

Filter ☐
Boiled ☐
Instant ☐
Decaffeinated ☐
Other ☐ Please specify_____

18) Do you add salt to the food at the table?

YES ☐
NO ☐

19) How many meals do you usually take per day?

0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 or more ☐

20) How many snacks do you usually take per day? (Snacks are food consumed in between meals, for example: chips, fruit, nuts, biscuits, etc.)

0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 or more ☐

21) How many meals do you usually take out of home?

0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 or more ☐

22) Have you had anything to eat for breakfast this morning?

YES ☐ What? _____
NO ☐

23) Do you suffer from any medical condition requiring a special diet?

YES ☐ Which one? _____
NO ☐

Appendix 2.1. Life-style questionnaire and background data.....continue...

24) Are you vegetarian?

YES ☐

NO ☐

25) Do you eat the following food?

	Yes	No
Red meat	☐	☐
Chicken	☐	☐
Fish or seafood	☐	☐
Eggs	☐	☐
Milk and milk products	☐	☐

26) Do you take any sort of medication? (Including contraceptives)

YES ☐ If yes, Please specify _____

NO ☐

27) Do you take vitamin or mineral supplements?

YES ☐ If yes, Please specify _____

NO ☐

28) How often have you gone on a diet during the last year? By diet we mean changing the way you eat so you can lose weight.

Never ☐

1 to 4 times ☐

5 to 10 times ☐

more than 10 times ☐

I am always dieting ☐

29) Have you ever tried to lose weight with any of the following?

	Yes	No
Fasting	☐	☐
Fad diets (for example Moon diet, Atkin's diet, etc.)	☐	☐
Eating less	☐	☐
Vomiting	☐	☐
Laxatives	☐	☐
Diuretics (water pills)	☐	☐
Slimming pills (for example: amphetamines, homeopathic pills)	☐	☐
Meal replacement (for example Stop Calorie)	☐	☐
Skipping meals	☐	☐
Decreasing fat intake (for example: giving up butter, confectionery, etc.)	☐	☐
Increasing exercise	☐	☐
Other _____	☐	☐

Appendix 2.1. Life-style questionnaire and background data……..continue…

30) Which statement describes you best?

I would like to put on weight ☐

I would like to lose weight ☐

I am happy with my weight as it is ☐

31) Have you ever been diagnosed with Anorexia Nervosa?

YES ☐ If yes, please specify_____

NO ☐

32) Have you ever been diagnosed with Bulimia Nervosa?

YES ☐ If yes, please specify_____

NO ☐

33) How old are you?_____

34) Which adults do you live with?

Mother and father ☐

Mother only ☐

Father only ☐

Mother and step father ☐

Father and step mother ☐

Foster parents ☐

Other ☐ Specify _____

35) Have you started your periods yet?

YES ☐

NO ☐ *go to question 35*

36) If so, at what age did you get your first period?_____

37) How often do you menstruate?

Don't menstruate ☐

Once per month ☐

Every 2 months ☐

Every three months ☐

Other ☐ Specify_____

38) How much do you agree or disagree with these statements?

	Disagree	Not sure	Agree
I am in charge of my health	☐	☐	☐
If I keep healthy I have just been lucky	☐	☐	☐
If I take care of myself I will stay healthy	☐	☐	☐
Even if I look after myself I can still easily fall ill	☐	☐	☐

Appendix 2.1. Life-style questionnaire and background data…..continue…

39) When choosing what to eat, do you consider your health?

Never ☐
Sometimes ☐
Quite often ☐
Very often ☐
Always ☐

40) How much was your
Maximum weight _____ (kg)?
Minimum weight_____ (kg)?

41) Please complete your name and surname_____

COMMENTS (please add any comments you would like to make)

_____
_____
_____
_____
_____
_____
_____
_____
_____
_____
_____

Please check that you have answered every question.

Thank you for giving the time to fill in this questionnaire!

If you have any queries, do not hesitate to contact Valeria Matzkin Tel: 48011282

Appendix 2.2. Food diary sheet

Name: **Date:** /2001

DAY 1				
TIME	DESCRIPTION OF FOOD OR DRINK	QUANTITY Portion size	Where?	With who?

Appendix 2.3. Portion size guide

Food item	Serving			
	Medium	Small	Large	Very large
Milk	**1 glass (200 cm³)**	½	1 and ½	2
Milk pudding, yoghurt	**1 container**	½	1 and ½	2
Cottage cheese	**2 tablespoon**	1	3	4
Cheese	**1 portion the size of a medium match box**	½	1 and ½	2
Red meat, pork, lamb, liver, kidney, fish	**1 slice the size of the palm of your hand**	½	1 and ½	2
Chicken	**1 piece**	½	1 and ½	2
Tuna	**½ cup (100 cm³)**	¼	¾	1
Hot dog	**2 pieces**	1	3	4
Hamburger	**1**	½	1 and ½	2
Ham	**2 thin slices**	1	3	4
Wine, juice	**1 glass (200 cm³)**	½	1 and ½	2
Beer	**1 can**	½	1 and ½	2
Mate	**4 mates**	2	6	8
Coffee, tea	**1 cup (200 cm³)**	½	1 and ½	2
Sugar, marmalade, caramel, honey	**1 tablespoon or 4 teaspoons**	½ or 2	1 and ½ or 6	2 or 8
Bread	**2 slices**	1	3	4
Biscuits, crackers	**4**	2	6	8
Confectionery	**1 piece**	½	1 and ½	2
Soup	**1 cup (200 cm³)**	½	1 and ½	2
Pastries	**1**	½	1 and ½	2
Sweet or vegetable cake	**1 portion**	½	1 and ½	2
Chocolate, cereal bar	**1 medium bar**	½	1 and ½	2
Sweets (caramels)	**4**	2	6	8
Ice cream	**1 cup (200 cm³)**	½	1 and ½	2
Chips	**1 plate (23 cm diameter)**	½	1 and ½	2
Carrot, pepper, beetroot, potato, onion, tomato	**Fresh: 1 medium Cooked: ½ cup**	½ or ¼	1 and ½ or ¾	2 or 1

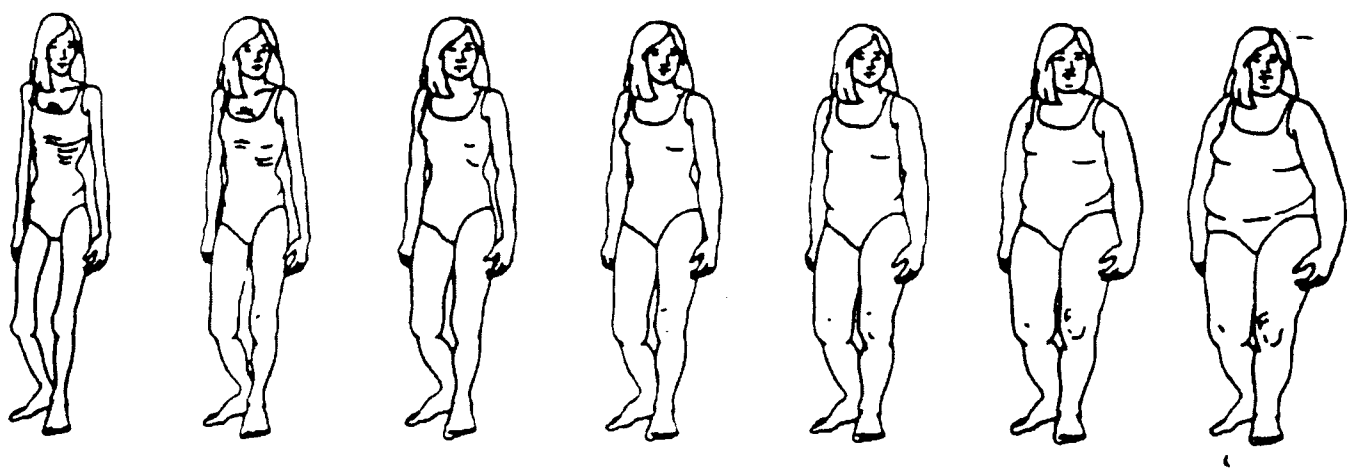
Appendix 2.3. Portion size guide

continue...

Food item	Serving			
	Medium	Small	Large	Very large
Beans, broccoli, cauliflower, spinach, greens, cabbage, lettuce, pumpkin, zucchini	**1 cup (200 cm³)**	½	1 and ½	2
Corn	**1 medium**	½	1 and ½	2
Grape fruit	½	¼	1	1 and ½
Banana, apple, orange mandarin, peaches	**1 medium**	½	1 and ½	2
Plums	**2 medium**	1	3	4
Melon	**1 slice**	½	1 and ½	2
Grapes	**1 cup (200 cm³)**	½	1 and ½	2
Dehydrated fruit	**2 medium**	1	3	4
Nuts	**1 handful**	½	1 and ½	2
Butter, margarine, oil, cream, mayonnaise, ketchup, seafood sauce, mustard	**2 tablespoon**	1	3	4
Rice, spaghetti, lentils, chickpeas, lasagne, gnocchi.	**1 plate (23 cm diameter)**	½	1 and ½	2
Pizza	**2 slices**	1	3	4
Soya burger	**1**	½	1 and ½	2
Egg	**1**	½	1 and ½	2

Appendix 2.4. Figure rating scale for adolescents and adults

Stunkard and colleagues (1983)



Which picture looks most like you?

Which picture shows the way you want to look?

Appendix 2.5. Manual of anthropometric measurements

The measurements were taken with the participants wearing a minimal amount of clothes (underwear). Each measurement was taken by the same observer twice in order to obtain more accurate results and to look for the intra-observer variances. The values were then averaged. Measurements were taken on the left side of the body.

1) Height (m) was measured with subjects standing straight, back to a portable stadiometer. Head, hips and heels were in contact to the stadiometer. The head was adjusted so that the Frankfort plane (the inferior border of the eye at the same plane as the external auditory meati) was horizontal. Bare feet were kept parallel, with the heels together. An horizontal surface was held on the top of the head to obtain the reading. The measurement was taken after gentle expiration.

2) Weight (kg) was measured in bare feet with subjects standing straight and wearing minimal clothing. A digital personal weighing scale was used. Measurements were recorded to the nearest 0.5 kg.

3) Waist circumference (cm) was taken midway between the lateral lower ribs and iliac crests while subjects were standing erect. The position was around the level of the umbilicus. Subjects were asked not to tuck their stomachs in and the measurement was recorded after gentle expiration. Subjects were required to keep the arms at the sides, feet together and with the weight equally divided over both legs. A flexible steel tape was used. No clothes were around the waist area. Measurements in cm were recorded to the nearest 0.5 cm.

4) Hip circumference (cm) was taken at the point yielding the maximum circumference over the buttocks. Subjects stood erect with their arms at their sides and their feet together.

5) Mid-Arm circumference (cm): was taken at the midpoint of the upper arm, with the arm hanging relaxed. It was taken half way down the arm between the tip of the acromion and the tip of the alecronon. The arm was bent at the elbow towards the shoulder in a straight line, this distance was measured and the half way-point marked horizontally with a pen. A flexible steel tape was placed around the arm gently but firmly to avoid compression of the soft tissues.

6) Wrist circumference (cm): A flexible steel tape was used to measure wrist circumference. Body frame (height/wrist circumference) could be estimated from this measurement.

7) Skinfold thinness: biceps skinfold (mm), triceps skinfold (mm), suprailiac skinfold (mm) and subscapular skinfold (mm). Skinfold thickness was assessed as Durnin and Raham (1967) have explained. A skinfold calliper recorded the measurements, with the subjects standing. The calliper was taken with the right hand. The skinfold was picked up firmly between the thumb and forefinger of the left hand and pulled away slightly from the underlying tissue. The calliper was applied to the fold a little below the pinch point. At the moment that the calliper jaws were applied to the skinfold, the thumb and the forefinger were removed from the calliper, so that the jaws could exert their full pressure. The pinch on the left hand was maintained and a reading taken after 2 seconds. The measurement was recorded to the nearest 0.2 mm. The dial on the calliper moved in a anti-clockwise direction.

- Biceps skinfold was taken at the midpoint of the upper arm, with the arm hanging relaxed. It was recorded half way down arm between the tip of the acromion of the scapula and the tip of the olecranon of the ulna, at the same point where the arm circumference was taken. The fold was measured 1 cm above the level of the caliper. The measurement was taken in the biceps muscle parallel to the arm.

- Triceps skinfold was recorded at the midpoint of the upper arm, with the arm hanging relaxed. It was taken half way down arm between the tip of the acromion of the scapula and the tip of the olecranon of the ulna, at the same point where the arm circumference was taken. The fold was measured 1 cm above the level of the calliper. This was estimated in the triceps muscle parallel to the arm.
- Subscapular skinfold was taken just below the angle of the scapula. Subjects were standing with the arm and shoulder relaxed. The fold was picked up slightly inclined in the natural cleavage line of the skin.
- Suprailiac skinfold was measured with the subject breathing gently. It was measured just above the crest in the mid-axillary line.

8) Anthropometric calculations: Total body fat was estimated as a percentage of body weight, taking skinfold measurements at mid-biceps, mid-triceps and iliac and subscapular sites. BMI (kg/m2) was also calculated.

The calculation of percentage of body fat involved the conversion of the total skinfold, measured in mm, into a logarithmic value. This value was then replaced for the value of the body density in the Siri's equation (Siri, 1956). For example, if the total skinfold thickness of a girl was 25 mm, the logarithmic value of this is 1.347. To obtain body density these values are applied to the formula:

Body density=1.1369-0.0598 X

Body density=1.1369-0.0598 (1.347)

Body density=1.057

If this value of body density is replaced in the Siri's equation:

Fat (%)=[(4.95/density)-4.5] 100

Fat (%)=[(4.95/1.057)-4.5] X100

Fat (%)=18.3

Appendix 2.6. Percentage of inadequate houses by neighbourhood, 1991, Buenos Aires city.

Neighbourhood	Inadequate houses*
Agronomía	2.6
Almagro	3.8
Balvanera	4.7
Barracas	16.9
Belgrano	1.4
Boca	17.4
Boedo	4.9
Caballito	**1.7**
Coghlan	2.5
Colegiales	2.3
Constitución	7.4
Chacarita	4.9
Flores	3.8
Floresta	3.0
Liniers	2.6
Matadero	3.3
Monte Castro	2.4
Monserrat	7.6
Pompeya	10.6
Nuñez	2.3
Palermo	2.2
Parque Avellaneda	11.7
Parque Chacabuco	3.5
Parque Patricios	6.0
Paternal	3.9
Recoleta	2.5
Retiro	5.4
Saavedra	3.4
San Cristóbal	5.8
San Nicolás	5.2
San Telmo	7.5
Velez Sársfield	2.5
Versalles	2.0
Villa Crespo	3.0
Villa del Parque	1.9
Villa Devoto	2.2
Villa General Mitre	3.2
Villa Lugano	**14.5**
Villa Luro	3.1
Villa Ortúzar	3.4
Villa Pueyrredón	2.7
Villa Real	2.9
Villa Riachuelo	4.7
Villa Santa Rita	2.2
Villa Soldati	14.0
Villa Urquiza	2.7
Zona Portuaria	51.3
Total	4.3

* Accommodation includes houses, hotels, hostels, mobile homes and cabins (cabin in Argentina implies a structure of corrugated metal, usually self-built).
Source: Dirección General de Estadísticas y Censos, Buenos Aires, Serie N 1, INDEC, Argentina. Anuario Estadistico de la ciudad de Buenos Aires.

Appendix 2.7. Letter to Schoolgirls' parents

Date:

Dear parent,

We are writing to request the participation of your daughter in a research project that the school is undertaking in collaboration with King's College London.

Young girls are usually preoccupied with their weight and body shape. Dissatisfaction with appearance often leads to dieting, which puts them at risk of nutritional deficiencies, eating disorders and other long-term health problems. The aim of my study is to explore attitudes and behaviours in schoolgirls with reference to chronic diseases such as cardiovascular disease and osteoporosis.

The research project will involve asking girls some questions about eating, dieting, exercise pattern, tobacco smoking, etc. The questionnaires will take less than thirty minutes to complete. I will also be taking their weight, height, waist and hip circumference, skinfold thinness (measurement of body fat), blood pressure, and asking some questions about body image. The girls will also have to complete a food diary, describing what they ate and drank for seven consecutive days.

This study could contribute to have a picture of the risk factors for chronic diseases among schoolgirls from Buenos Aires. All the information we obtain will be treated confidentially. If you decide to withdraw your daughter from the research project, please let the School or myself know about your decision. In addition to your agreement, consent will be sought individually from each girl.

I would like to thank you in advance for your help.

Yours sincerely,

Valeria Matzkin

Appendix 2.8. Photographs of anthropometric fieldwork in the EMEM 4 school

Appendix 4.1. Patient psychological and medical clinical record

1. Date:
2. Name of patient:
3. Sex:
4. Nationality:
5. Age/Date of birth:
6. Address/Telephone:
7. Marital status:
8. Occupation:
9. Schooling:
10. How did you become aware of your illness? TV, Radio, Newspaper, Magazine, Book, Friends, Neighbours, Conferences, Other
11. Was your birth? Normal, Caesarean, Forceps
12. Did you need artificial feeding? (Yes-No)
13. Do you suffer with problems of? the heart, the liver, the kidneys, the lungs, the bones, dermatological problems, gland problems, gynaecological problems, digestive problems, mental problems, nervous system
14. Do you have problems? Urinating, Defecating, Sleeping, Sexual
15. Do you smoke tobacco? (Yes-No)
16. Do you smoke? Pipe, Black cigarettes, White cigarettes
17. How much do you smoke per day? 1 to 5 cigarettes, 6 to10 cigarettes, 11 to 20 cigarettes, More than 20 cigarettes
18. Do you drink wine? (Yes-No)
19. How much per day? ½ glass, 1 glass, 2 glasses, More than 2 glasses
20. Do you drink distilled drinks? (Yes-No) Which one/s?
21. Do you consume drugs? (Yes-No) Which one/s?
22. Are you allergic to any food? (Yes-No) Which one/s?
23. How often do you eat?
24. Which are the foods you like the most?
25. Which are the foods you do not like?
26. Do you eat? Standing? Sitting? Slowly? Rapidly?
27. How much time do you spend on lunch or dinner?

Appendix 4.1. Patient psychological and medical clinical record

1. Date:
2. Name of patient:
3. Sex:
4. Nationality:
5. Age/Date of birth:
6. Address/Telephone:
7. Marital status:
8. Occupation:
9. Schooling:
10. How did you become aware of your illness? TV, Radio, Newspaper, Magazine, Book, Friends, Neighbours, Conferences, Other
11. Was your birth? Normal, Caesarean, Forceps
12. Did you need artificial feeding? (Yes-No)
13. Do you suffer with problems of? the heart, the liver, the kidneys, the lungs, the bones, dermatological problems, gland problems, gynaecological problems, digestive problems, mental problems, nervous system
14. Do you have problems? Urinating, Defecating, Sleeping, Sexual
15. Do you smoke tobacco? (Yes-No)
16. Do you smoke? Pipe, Black cigarettes, White cigarettes
17. How much do you smoke per day? 1 to 5 cigarettes, 6 to10 cigarettes, 11 to 20 cigarettes, More than 20 cigarettes
18. Do you drink wine? (Yes-No)
19. How much per day? ½ glass, 1 glass, 2 glasses, More than 2 glasses
20. Do you drink distilled drinks? (Yes-No) Which one/s?
21. Do you consume drugs? (Yes-No) Which one/s?
22. Are you allergic to any food? (Yes-No) Which one/s?
23. How often do you eat?
24. Which are the foods you like the most?
25. Which are the foods you do not like?
26. Do you eat? Standing? Sitting? Slowly? Rapidly?
27. How much time do you spend on lunch or dinner?

Appendix 4.2. Control subjects: background and lifestyle questionnaire

1) Date: _____
2) Name: _____
3) Date of birth _____
4) What is your nationality?_____
5) Please complete your address and telephone number?

_____

6) Marital status

Single ☐
Married ☐
Living with partner ☐
Divorced ☐
Widow ☐
Other ☐ Please specify_____

7) What is your current occupation?

_____

8) What is your father's occupation?

_____

9) What is your mother's occupation?

_____

10) What is your educational background?

_____

11) Was your birth?

Normal ☐
Caesarean ☐
Forceps ☐
Other ☐

12) Did you need artificial feeding?

Yes ☐
No ☐

13) Do you suffer with problems of?

The heart ☐
The liver ☐
The kidneys ☐
The lungs ☐
The bones ☐
Dermatological problems ☐
Gland problems ☐
Gynaecological problems ☐
Digestive problems ☐
Mental problems ☐
Nervous system ☐

Appendix 4.2. Control subjects: background questionnaire **continue...**

14) Do you have problems?

Urinating ☐

Defecating ☐

Sleeping ☐

Sexual ☐

15) Do you smoke tobacco?

Yes ☐

No ☐

16) If yes, do you smoke?

Pipe ☐

Black cigarettes ☐

White cigarettes ☐

Other ☐

17) How much do you smoke per day?

1 to 5 cigarettes ☐

6 to10 cigarettes ☐

11 to 20 cigarettes ☐

More than 20 cigarettes ☐

18) Do you drink wine?

Yes ☐

No ☐

19) How much per day?

½ glass ☐

1 glass ☐

2 glasses ☐

More than 2 glasses ☐

20) Do you drink spirits?

Yes ☐ Which one/s?_____ How much?_____

No ☐

21) Do you consume drugs?

Yes ☐ Which one/s? _____

No ☐

22) Are you allergic to any food?

Yes ☐ Which one/s? _____

No ☐

23) How often do you eat? (hours)

_____

Appendix 4.2. Control subjects: background questionnaire **continue...**

24) Are you vegetarian?

YES ☐

NO ☐

25) Do you eat?

	Yes	No
Red meat	☐	☐
Chicken	☐	☐
Fish or seafood	☐	☐
Eggs	☐	☐
Milk products	☐	☐

26) Which are the foods you like the most?

_____

27) Which are the foods you do not like?

_____

28) Do you eat?

	Yes	No
Standing	☐	☐
Sitting	☐	☐
Slowly	☐	☐
Rapidly	☐	☐

29) How much time do you spend on lunch or dinner? (minutes/hours)

_____

30) Do you consume high calorie food in short period of time? (less than 2 hours)

Yes ☐

No ☐

31) Do you finish these episodes with abdominal pain, sleep or inducing vomit?

Yes ☐

No ☐

32) Have you ever tried achieving weight loss with the use of?

	YES	NO
Strict diets	☐	☐
Vomiting	☐	☐
Diuretic	☐	☐
Laxative	☐	☐
Slimming pills	☐	☐

33) Have you had any weight fluctuation of more than 5 kg.?

Yes ☐

No ☐

34) Are you conscious that your food pattern is abnormal and are you scared of not being able to stop eating voluntarily?

Yes ☐

No ☐

35) Are you scared of putting on weight?

Yes ☐

No ☐

36) Does this fear decrease as your weight decreases?

Yes ☐

No ☐

37) Did you lose more than 25 kg of your original weight?

Yes ☐

No ☐

38) Did your menstrual periods stop?

Yes ☐

No ☐

39) How often do you menstruate?

Don't menstruate ☐

Once per month ☐

Every 2 months ☐

Every 3 months ☐

Other ☐ Please specify _____

40) Are you pregnant or suspect that you are pregnant?

Yes ☐

No ☐

41) Have you ever been hospitalised because of AN?

Yes ☐

No ☐

42) Have you ever-received psychiatric, medical or nutritional treatment?

Yes ☐ Which one/s? For how long?_____

No ☐

43) Do you take any medication?

Yes ☐ Which one/s? _____

No ☐

44) Do you practise any sport?

Yes ☐ Which one/s? _____

No ☐

45) How many times a week do you exercise?

_____

46) How can you define your study performance?

Bad ☐

Regular ☐

Good ☐

Very good ☐

47) Do you know any person suffering from Anorexia Nervosa?

Yes ☐

No ☐

48) Do you have any member in your family with a history of? (Please fill in the space with a cross)

	Grandparents	Parents	Sisters or brothers	Aunts or Uncles
Diabetes				
Obesity				
Atherosclerosis				
High Cholesterol				
Hypertension				
Malformations				
Tuberculosis				
Neurological changes				
Epilepsy				
Haemophilia				
Depression				
Anorexia Nervosa				
Bulimia Nervosa				
Other				

COMMENTS (please add any comments you would like to make)

Please check that you have answered every question.

Thank you for taking the time to fill in this questionnaire!

If you have any queries, do not hesitate to contact Valeria Matzkin
Tel: 48011282.

Appendix 4.3. Information sheet for patients

King's College London-ALUBA

Title of the project

Risk factors for cardiovascular disease and osteoporosis in patients with Anorexia Nervosa and in schoolgirls

Explanation

We would like you to participate in this research project. You should not take part if you do not wish to do so and, if you decide to take part, you are free to withdraw at any time without explanation. If you decide not to take part your treatment will not be affected by your decision. The aim of the study is to find out if individuals suffering from Anorexia Nervosa have more risk factors of diseases of the arteries and the bones than healthy subjects. For this we will test blood levels of cholesterol, cortisol, glucose, antioxidants, essential fatty acids and an amino acid called homocysteine. This study will contribute to a better understanding of abnormalities that occur in people suffering from this eating disorder. In addition, the results of this study could lead to therapeutic measures being adopted in order to prevent chronic diseases.

In order to participate in the study you need to be a woman, 15 years old or older, not suffer from Bulimia Nervosa and you must not be pregnant. You will be given an appointment at the ALUBA (Udaondo Hospital), which will last around one hour. We will ask you to come to hospital having fasted for nine hours (no food and drinks, except water, from 10 o'clock the night before). A phlebotomist will take 20 millilitres of blood from you. We will also record your blood pressure, weight and height. You will be asked to ask a written consent form to participate. We will repeat these measurements after a period of three months.

All the information you give us will be treated confidentially.

If you have any queries please do not hesitate to contact any medical doctor in the ALUBA.

Appendix 4.4. Information sheet for controls

King's College London- ALUBA

Title of the project

Risk factors for cardiovascular disease and osteoporosis in patients with Anorexia Nervosa and in schoolgirls

Explanation

We would like you to participate in this research project. You should not take part if you do not wish to do so and, if you decide to take part, you are free to withdraw at any time without explanation. The aim of the study is to find out if individuals suffering from Anorexia Nervosa exhibit more risk factors of diseases of the arteries and the bones than healthy subjects. To complete this study we need **healthy volunteers** of a normal weight to participate as the control group. If you wish to volunteer we would test blood levels of cholesterol, glucose, cortisol, antioxidants, essential fatty acids and an amino acid called homocysteine. We will also investigate behaviours through the use of questionnaires. This study would contribute to a better understanding of abnormalities occurring in people suffering from this eating disorder. In addition, the results of this study could lead to therapeutic measures being adopted in order to prevent chronic diseases.

In order to participate in the study, you will need to be a woman, 15 years old or older, not suffer or had suffered from Anorexia Nervosa, Bulimia Nervosa, Diabetes, Hypertension, Liver or Kidney diseases and you must not be pregnant. You will be given an appointment at the ALUBA (Udaondo Hospital), which will last around one hour. We will ask you to come to hospital having fasted for nine hours (no food and drinks, except water, from 10 o'clock the night before). A phlebotomist will take 20 millilitres of blood from you. We will also ask you to complete a questionnaire about your background and lifestyle and a consent form to participate. In addition, we will measure your weight, height and blood pressure. All these measurements will be repeated within a month.

All the information you give us will be treated confidentially. If you have any queries please do not hesitate to contact Valeria Matzkin on 48011282.

Appendix 4.5. Consent form

Title of Project:

Risk factors for cardiovascular disease and osteoporosis in subjects with Anorexia Nervosa and in schoolgirls

The participant or key carer should complete the whole of this sheet himself/herself. (Please cross out as necessary)

1. Have you been asked to consent for yourself or on behalf of someone else Self/Other
2. If your answer to the above is "other", please give the name of the person for whom you are consenting.....
3. Have you read the Information Sheet for Patients/ Healthy Volunteers? Yes/No
4. Have you had an opportunity to ask questions and discuss this study? Yes/No
5. Have you received satisfactory answers to all of your questions? Yes/No
6. Have you received enough information about the study? Yes/No
7. Who have you spoken to? Dr/Mr/Ms .....
8. Do you understand that you are free to withdraw from the study at any time, without having to give a reason for withdrawing? Yes/No
9. Do you agree to take part in this study? Yes/No

Signed ..... Date .....

(NAME IN BLOCK LETTERS) .....

(Relationship to the subject if not the participant: parent/guardian/other carer)

INVESTIGATOR'S STATEMENT

I confirm that I have carefully explained the nature, demands and foreseeable risks of the proposed study to the patient/volunteer.

Signed ..... Date .....

(NAME IN BLOCK LETTERS) .....

Appendix 4.6. Fatty acids and vitamin methodology

1. Determination of plasma fatty acids

Reference: Lepage and Roy (1986)

Materials

Internal Standard (50 μg pentadecanoic acid/ml)

Hexane (puriss)

Acetyl Chloride

Methanol AR

Toluene AR

60g Potassium Carbonate/L water

Hewlett Packard GC6890, fitted with and FID, equipped with a 25m BP75 column (SGE)

Internal standard was made at a concentration of 50μg pentadecanoic acid/mL in methanol: toluene (4:1 by volume). 5mL of internal standard solution (in methanol) is added to 75mL of methanol. The solution is made up to 100mL by the addition of toluene. 10 mL acetyl chloride solution was added dropwise, with stirring, to the internal standard solution. 0.1mL plasma was pipetted into a Teflon lined screw capped tube (16x25mm) and 2.2mL of internal standard mixture was added. Tubes were flushed with nitrogen, sealed, heated for 2 hours at 60°C, and then allowed to cool. 5 mL of 6% aqueous potassium carbonate was added. Tubes were centrifuged at 3000rpm for 20 minutes, the upper phase was collected and transferred with a microsyringe into a GLC vial. 1 μL was injected into the GLC, equipped with a 25m BP75 column (SGE). Individual fatty acids were identified by reference to standards obtained from Sigma (fatty acid methyl ester standard 189-1,189-2, 189-3) and a secondary reference standard of MaxEPA (Seven Seas Ltd, Hull, UK) for long-chain n-3 fatty acids.

Operating parameters

Column: BP75 SGE

Carrier gas: Hydrogen

Make up gas: Nitrogen

Injection volume: 1μL

Injection mode: Split 50:1

Inlet temperature: 250°C

Detector temperature: 250°C

Oven programme: Initial temperature 180°C, held for 2 minutes, then ramped 3°C/minute and held for 10 minutes.

Column flow: 0.6mL/minute

Data acquisition rate: 20Hz

2. Simultaneous Determination of Beta-carotene, Vitamin A and Vitamin E in plasma by HPLC.

Reference: Thurnham et al (1985)

Materials and settings

Internal standard: tocopherol acetate (40 μmol /L ethanol)

Retinol

Tocopherol

Beta-carotene

HPLC grade n-heptane (BDH Chemicals Ltd, Poole, Dorset, UK.)

Hexane puriss grade

C18 reverse phase column Spherisorb ODS-2 100 x 4.6mm 3mm

UV detection 292 nm and 435nm

20 μl microlitre sample loop

Settings air gap 5, sample 60 into loop 50

Mobile Phase 47% methanol, 47% acetonitrile, 6% chloroform (all HPLC grade)

Flow rate 1 mL/min

Appendix 4.6. Fatty acids and vitamin methodology **continue....**

Sample preparation

0.25mL plasma was accurately pipetted into a clean glass test tube. 0.5mL internal standard solution was added and the tube vortex mixed for 60 seconds. 1mL of heptane was added and the tube vortex mixed thoroughly for 2.5 min and then centrifuged at 2500 RPM for 10 minutes at 20°C. The upper phase (0.7mL) was carefully collected with a glass Pasteur pipette into a clean glass test tube and the solvent removed under nitrogen at 40°C. The extract was then redissolved in 100μL chloroform enriched mobile phase (43:43:16) and kept in the dark on ice prior to injection onto the column.

Standard preparation

A standard curve was prepared from stock solutions of the following concentrations. The concentrations are checked using their UV absorbance on dilutions using the molecular extinction coefficient. Stock solutions were stored in the dark in a spark proof fridge

Retinol 10 mg/dL and 100 μg/mL in ethanol

α-tocopherol 120mg/dL and 1.2 mg/mL in ethanol

β-carotene 5mg/25mL and 200 μg/mL in hexane

α-tocopherol acetate 189mg/dL in ethanol

A working standard mixture in 10mL ethanol was prepared daily to give final concentrations of

50 μg/dL for retinol

1.2 mg/dL for α-tocopherol

40μg /dL for β-carotene

To achieve this the stock solutions were first diluted by 1 in 10 with ethanol. Appropriate amounts of each of these dilutions (0.5mL, 1mL and 0.2mL for retinol, α-tocopherol and β -carotene respectively) and made up to 10mL in a volumetric flask with ethanol. The working solutions were made up in amber volumetric flasks.

Appendix 4.6. Fatty acids and vitamin methodology continue....

The working internal standard solution (40μmol/L) of 1.89mg/dL was prepared by diluting the stock solution 1 in 100mL in ethanol.

A standard curve was prepared by pipetting the following volumes (mL).

Retinol	**α-tocopherol**	**β - carotene**	**Volume Standard**	**Internal Standard**
100μg/dL (3.49)*	2.4mg/dL (55.8)	80 μg/dL (1.49)	0.5	0.5
50 μg/Dl (1.745)	1.2mg/dL (27.9)	40 μg/dL (0.745)	0.25	0.5
25 μg/dL (0.875)	0.6mg/dL (13.95)	20 μg/dL (0.3725)	0.125	0.5

*Values in parenthesis are equivalent μmol/L concentrations

The samples were dried down under nitrogen and re-dissolved in 250μL chloroform enriched mobile phase (43:43:16) and injected onto the HPLC column. Standard curves of peak area of the analyte/peak area of the internal standard vs. concentration were plotted for each antioxidant and the concentrations in the sample are determined from this.

The normal ranges in μmol/L are

Retinol 2.2 μg/dL (1.4-3.2 mmol/l)

β-carotene 0.29 μg /dL (0.07-0.66 mmol/l)

α-tocopherol 27.1 mg/dL (15.1 – 43.1 mmol/l)

Appendix 5.1. Questionnaire for treatment assessment

General section

1) Date:

2) Name of the Institution:

3) Which of the following disorders do you treat?

Anorexia Nervosa ☐
Bulimia Nervosa ☐
Eating Disorder Not Otherwise Specified ☐
Obesity ☐
Other ☐ Please specify---------

4) How many patients with Anorexia Nervosa (AN) are you currently treating?

5) How many admissions of AN do you have per month?

6) Which of the following programmes for patients with AN do you offer?

Hospitalisation ☐
Day patient programme ☐
Out patient programme ☐
Other ☐ Please specify----------

7) What is the average length of treatment for the following programmes of AN? (months)

Hospitalisation _____
Day patient programme _____
Out patient programme _____
Other/Specify _____

8) What criteria of diagnosis do you use for Anorexia Nervosa?

9) What do you consider are the three most significant short-term risks of Anorexia Nervosa?

1)_____
2)_____
3)_____

10) What do you consider are the three most significant long-term risks of Anorexia Nervosa?

1)_____
2)_____
3)_____

11) Number the following in the order of what you consider are the priorities for treatment?

- Restoration of weight loss
- Normalise clinical parameters (such as electrolyte balance, blood pressure, body temperature, etc.)
- Help the patient in establishing personal relationships
- Treat the symptoms of depression
- Induce menstruation
- Promote a balanced diet with adequate intake of carbohydrates, protein and fat
- Encourage the intake of frequent meals (for example: 4 meals and 2 snacks)
- Eliminate abnormal eating behaviours (vomiting, laxative, diuretic and slimming pill use)
- Eliminate substance use (tobacco, alcohol and illegal drugs)

Appendix 5.1. Questionnaire for treatment assessment **continue...**

12) Do you have set protocols on the following areas to treat patients that all the professionals follow?

	No	Yes	Specify
Drug prescription	☐	☐	_____
Dietary advice	☐	☐	_____
Clinical testing	☐	☐	_____
Other	☐	☐	

13) Do you test blood for levels of total cholesterol on admission?

Yes ☐ Please specify_____

No ☐

14) Do you test for total cholesterol levels during treatment?

Yes ☐ How many times?_____

No ☐

15) If yes, do you prescribe any of the following to treat the hypercholesterolemia in patients with Anorexia Nervosa?

	No	**Yes**	**Specify**
Medication	☐	☐	_____
Dietary plan	☐	☐	_____
Exercise	☐	☐	_____
Other	☐	☐	_____

16) Do you ask for information about hypercholesterolemia in the patient's parents?

Yes ☐ Please specify_____

No ☐

17) Do you test for cardiovascular abnormalities in patients with Anorexia Nervosa?

Yes ☐ Which tests?_____

No ☐

18) In what percentage of patients do you see the following problems?

	Less than 25%	**25 to 50%**	**51 to 75%**	**More than 75%**
Blood cholesterol above 200 mg/dl	☐	☐	☐	☐
Arrhythmia	☐	☐	☐	☐
Hypertension	☐	☐	☐	☐
Hypotension	☐	☐	☐	☐
Chest pain	☐	☐	☐	☐
Infarction	☐	☐	☐	☐
Bone fracture	☐	☐	☐	☐
Bone loss	☐	☐	☐	☐

19) Do you think that any of these cardiovascular abnormalities are a serious problem in Anorexia Nervosa?

Yes ☐

No ☐

20) Do you think that any of these bone abnormalities are a serious problem in Anorexia Nervosa?

Yes ☐
No ☐

21) Do you test for bone loss in Anorexia Nervosa?

Yes ☐ Which tests?_____
No ☐

22) Do you prescribe any of the following to treat bone loss in patients with Anorexia Nervosa?

	No	Yes	Specify
Medication	☐	☐	_____
Dietary plan	☐	☐	_____
Exercise	☐	☐	_____
Other	☐	☐	_____

Nutrition section

1) Do you give dietary advice to patients with Anorexia Nervosa?

Yes ☐
No ☐

2) If yes, to whom do you give the advice?

Patient ☐
Family ☐
Both ☐

3) If yes, who gives dietary advice?

Psychiatrist ☐
Psychologist ☐
Nurse ☐
Dietician ☐
Clinician ☐
Other ☐ Please specify _____

4) If yes, what dietary advice do you give?

At admission:
Later on in treatment:

5) Do you have standardised diet plan sheets for patients with Anorexia Nervosa?

Yes ☐ Can I have a copy of this? Yes/No
No ☐

6) Do you calculate energy requirements in patients with Anorexia Nervosa?

Yes ☐ How? _____
No ☐

7) Do you calculate macronutrient intake?

Yes ☐
No ☐

8) Do you advise patients to consume a specific amount of energy, specific proportions of carbohydrate, protein and fat?

Yes ☐
No ☐

9) If yes, what percentage from the total energy intake from:
Carbohydrate_____
Protein _____
Fat_____**are patients advised to consume?**

10) Do you give advice to patients with Anorexia Nervosa on the intake of the following?

	No	Yes	**What is the advice?**
Meat	☐	☐	_____
Milk and milk products	☐	☐	_____
Bread and cereals	☐	☐	_____
Fruit and vegetables	☐	☐	_____
Food containing sugar	☐	☐	_____
Food containing fat	☐	☐	_____
Vegetable oils	☐	☐	_____
Salt intake	☐	☐	_____
Diet products	☐	☐	_____

11) How many meals do you advise patients to have each day?
At admission:
Main meals _____
Snacks _____
Later on in treatment:
Main meals _____
Snacks _____

12) How do you make sure patients are eating the right amount of food?
Don't check ☐
Supervising meals ☐
Educating patients on portion sizes ☐
Other ☐ Specify_____

13) Do you set a target weight?
At admission:
Yes ☐ Specify_____
No ☐
Later on in treatment:
Yes ☐ Specify_____
No ☐

14) In your experience what proportion of patients achieve the following results?
Reach the weight target _____
Relapse _____

15) In your experience, do patients with Anorexia Nervosa become overweight (BMI>25 kg/m²) after refeeding?
Yes ☐
No ☐

16) If yes, what percentage of patients with Anorexia Nervosa become overweight (BMI>25 kg/m²) after refeeding?

Less than 25% ☐
26 to 50% ☐
More than 50% ☐

17) How do you manage patients with Anorexia Nervosa who become overweight after re-feeding?

Don't do anything ☐
Put on a low calorie plan ☐
Prescribe exercise ☐
Other ☐ Specify _____

18) Do you prescribe sip-food supplements to patients?

Yes ☐ which one/ones? _____
No ☐

19) If yes, when do you use sip-food?

_____

20) Do you prescribe vitamin/mineral supplements to patients?

Yes ☐ which one/ones? _____
No ☐

21) If yes, when do you use vitamin or mineral supplements?

_____

22) Is food supplemented to achieve high energy density and to rapidly recover weight loss? (For example adding extra cream, cheese, full fat milk, egg, oils, etc.)

Yes ☐ With which food? _____
No ☐

23) Are the dietary treatments for day and out patients different?

Yes ☐ In what aspect? _____
No ☐

24) Do you agree with patients being vegetarian?

Yes ☐
No ☐

25) Do you agree with patients being vegan?

Yes ☐
No ☐

26) Do you agree with patients smoking tobacco?

Yes ☐
No ☐

27) Do you advise patients to exercise?

Yes ☐ When? _____
For how long? _____
No ☐

28) Do you agree with patients consuming alcohol?

Yes ☐
No ☐

29) Do you agree with patients consuming coffee?

Yes ☐
No ☐

30) Do you agree with patients adding salt at the table?

Yes ☐

No ☐

31) Do you agree with patients consuming low fat products?

Yes ☐

No ☐

Comments: (please add any comments you would make)

Thank you for your time in completing this questionnaire!

All the information you provide us will be treated confidentially

- Notes missing blood total cholesterol, age at diagnosis, occurrence of vomiting, reported exercise, use of tobacco or BMI.
- Notes where total cholesterol concentrations were not determined by enzymatic methods.

Control group

Data on the healthy Argentinean population were taken from two studies carried out by Selles and colleagues (1997) and Coniglio and colleagues (1997) in the south of Buenos Aires. The studies of the south can be extrapolated to the city of Buenos Aires because they were all conducted in urban regions. In any event, no significant differences were found in blood total cholesterol concentrations between these studies and the study of Neuman and co-workers (1979) on healthy female subjects from central Buenos Aires. These studies are the most contemporary investigations on risk factors for cardiovascular diseases in the young female population of Argentina.

The inclusion and exclusion criteria for taking part in these studies are listed below:

Inclusion criteria

- The Argentinean data consisted of 216 subjects (162 women from 13 to 19 years and 54 women from 20 to 29 years), who were of comparable age and sex with the patient group.
- The youngest group consisted of healthy members of the general public attending medical health screening from secondary schools and the University of the South (Bahia Blanca, Buenos Aires). The older group were state employees, shop owners, housewives, etc., who voluntarily participated in cholesterol education campaigns in Viedma (Buenos Aires) (Personal communication with Coniglio, 2000).
- The participants fasted for 10 to 12 hours before the biochemical determinations. The samples were analysed for cholesterol, HDL and triglycerides (TG) by enzymatic

3.3. Results

3.3.1. Characteristics of patients

At the time of the investigation Aluba had around 8000 clinical notes. Most of these had a routine blood analysis (red blood cells, haemoglobin, haematocrit, uric acid, glucose, hepatogram (cholesterol, bilirubin, transaminases), proteinogram (albumin, globulin), thyroid hormones and urine analysis. Routine blood analyses were usually repeated every six or more months, although in general only one analysis was present in the oldest clinical notes. The most recent notes (from 1995 onwards) contained an admission interview with information about age, vomiting and other purgative methods, tobacco use and exercise. 50% of the lipid profile analysis in patients were carried out in the lab of the Gastroenterology Hospital and the other 50% in different labs.

A total of 321 clinical notes were reviewed. More notes than needed, according to the sample size calculation, were reviewed to allow comparison with the other components of the lipid profile (LDL), since many notes were found to omit these data. Data on the general Argentinean population included subjects younger than 29 years. Therefore, those patients older than 29 years were excluded from the study. This group accounted for only 3.1% of the total patient sample. Only 3 patients (N= 293) had fasting glucose values on admission greater than 120 mg/dl, which could be used as an indicator of diabetes. Blood lipids in these patients were within normal values and therefore they were not excluded from the analysis. None of the patients had T3 (N= 56) or T4 (N=55) levels on admission greater than reference values (80-220 ng/dl and 5-12 μg/dl respectively), which could indicate hyperthyroidism.

Patients on admission were, on average, 17.3 years (SD: 3.8, ranging from 10.0 to 29.0 N= 308). Age at onset of AN, which was assessed from a question where patients were asked at what age they started to exhibit symptoms, was on average 14.7 years (SD 2.8, range 5.0 to 24.0, N=295).

significant differences between the two determinations were observed in any of the other components of the lipid profile (LDL, HDL and triglycerides).

3.3.2. Comparisons between patients and controls

Subjects were divided into two groups (1. from 10 to 19 years old, and 2. from 20 to 29 years old) in order to compare cholesterol values with the general population. The general Argentinean population control group consisted of 162 healthy females from 10 to 19 years old (BMI: 19.0 kg/m^2, SD 2.6 N=76) and 54 women from 20 to 29 years old (BMI: 21.9 kg/m^2, SD 3.1 N=53). The sample size of the biochemical and anthropometric variables compared in patients and controls is shown in Figure 3.1.

Table 3.2: Lipid composition (mean ±) in patients with AN on admission and healthy population adjusted for BMI and age

Variables	Subjects younger than 20 years old			Subjects between 20 and 29 y old			Total		
	Patients	Healthy population	P value	Patients	Healthy population	P value	Patients	Healthy population	P value
TC (mg/dl)	189 ± 33 N= 230	168 ± 42 N=162	0.026	230 ± 40 N=78	172 ± 37 N=54	0.013	193 ± N=308	171 ± N=216	0.000
HDL (mgl/dl)	58 ± 16 N=78	48 ± 8 N=90	NS	59 ± 15 N=25	49 ± 12 N=54	NS	58 ± N=103	50 ± N=144	NS
LDL (mg/dl)	120 ± 41 N=74	88 ± 34 N=162	0.000	126 ± 41 N=24	109 ± 39 N=54	NS	123 ± N=98	96 ± N=216	0.000
TG (mgl/dl)	79 ± 43 N=66	105 ± 79 N=90	NS	95 ± 38 N=25	90 ± 49 N=54	NS	91 N=91	95 N=144	NS
TG/HDL	1.4 ± 0.9 N=50	2.1 ± 1.4 N=90	NS	1.7 ± 0.7 N=25	2.0 ± 1.4 N=54	NS	1.63 N=71	2.08 N=144	NS

*Univariate General Linear Model test

Table 3.3: Percent of patients with AN on admission and the healthy population with lipid risk factors by age group

Age	Average risk cut off points NCEP (1992, 2001)		Patients		Healthy population	P values[2]
< 20 years	TC ≥ 5.70 mmol/l	230	19.1	162	5.6	0.000
	LDL ≥ 3.75 mmol/	74	17.6	162	3.7	0.001
	HDL ≤ 1.15 mmol/l	78	15.4	90	37.8	0.001
	TG ≥1.70 mmol/l	66	9.1	90	4.4	NS
20 to 29 years	TC ≥ 5.70 mmol/l	78	26.9	54	11.1	0.021
	LDL ≥ 3.75 mmol/l	24	33.3	54	14.8	NS
	HDL ≤ 1.15 mmol/l	25	16.0	54	44.4	0.012
	TG ≥ 1.70 mmol/l	25	8.0	74	16.7	NS

*Pearson Chi² test

nervosa and controls. The present study showed that vomiting was used by a minority of patients (27%) and supported the findings of Bhanji and Mattingly (1981) who demonstrated that cholesterol concentrations and vomiting were unrelated.

The higher total cholesterol in patients with AN may also be a consequence of the elevated HDL. In this study no correlations were found between exercise and HDL concentrations, however patients might have underreported exercise levels. In addition, the finding that HDL levels were not different between patients and controls when adjusted for BMI, suggests that the elevated HDL in patients may be a consequence of the low body weight.

Thirty four percent of patients had hypercholesterolemia, according to the criteria of the NCEP (2001) to categorise elevated total cholesterol values. This observation is consistent with the study of Crisp (1965), who stated that it is predictable to find hypercholesterolemia in about a third of the patients. If young patients did not undertake treatment they would remain a high-risk group for cardiovascular disease in the future. As patients age they may be at greater risk of suffering from the consequences of increased blood cholesterol.

Studies on cholesterol measurement after treatment have been controversial. Blendis and Crisp (1968) found that concentrations of TC in patients with AN significantly decreased at a 2-month follow up (N=8). The vast majority of the studies (Chapter 1) reported cholesterol concentrations that were not significantly lower in patients at follow up (ranging from 1 to 10 months) compared with admission. However, in most of these studies there was a tendency for cholesterol decrease at follow up. Conversely, Umeki (1988) found higher values of cholesterol at discharge than at admission.

In the present study, total cholesterol concentrations decreased after a mean treatment period of 9 months. However, no differences were noticed in any of the other variables of the lipid profile. There are no studies in the existing literature re-measuring cholesterol concentrations after 10 months of treatment. Therefore, further follow up studies are needed in order to elucidate after what length of treatment levels

Chapter 4 - Follow up patients with AN

(Studies 3 and 4)

3.2.2.4. Statistics

Frequencies and descriptive statistics were required in order to examine the characteristics of both the patients and the control subjects and to test for normality. In order to investigate the differences in mean values (total cholesterol, LDL, HDL, Apo A, Apo B, triglycerides, hormones, fibrinogen, glucose, vitamins, homocysteine and essential fatty acids) between the two groups, the parametric t-test for independent groups was selected. Adjustments were made for confounding variables such as age, BMI and TC, using Univariate analysis of variance. Lipid-soluble vitamins were adjusted for TC because in the blood these vitamins are associated with lipoproteins and increased blood lipids may denote an apparent high vitamin status. Correlations were carried out by the Pearson test. For the biochemical variables, the level of significance was reduced to 0.01, rather than 0.05, in order to correct for multiple testing. Estradiol concentrations were logged to permit the use of parametric tests.

Declaration

The author of this thesis designed the study, carried out anthropometric and blood pressure studies and extracted the blood. The author also analysed vitamins and fatty acids at King's College London and participated in the analysis of fibrinogen at the University of Buenos Aires. All the other analyses were performed in the labs described above. The data were analysed by the author.

4.2.3.3. Biochemical analyses

Lipid profile, fibrinogen, homocysteine and glucose

Table 4.3 shows significant differences between patients and controls in TC, LDL, Apo B after adjusting for age and BMI (kg/m^2). Higher concentrations of TG, fibrinogen, cortisol and Apo A and lower concentrations of glucose and HDL were found in patients compared with controls; however, these differences were not significant. One patient on admission had TG concentrations above 400 mg/dl and LDL levels could not be calculated.

Restricting type patients showed higher cholesterol (200.8 mg/dl, SD 58.1) and LDL levels (122.2 mg/dl, SD 48.0) compared with the non-restricting type of patients (cholesterol: 172.3 mg/dl, SD 32.3 and LDL: 104.8 mg/dl, SD 24.8). However, these differences were not significant.

Table 4.3: Biochemical variables associated with cardiovascular risk in patients on admission and controls adjusted for age and BMI (kg/m^2) (Mean and SD)

Variables	Patients with AN (N= 30)		Control subjects (N= 30)		P values
	Mean	SD	Mean	SD	(95 confidence nterval)
TC (mg/dl)*	191.3	52.2	160.0	24.0	0.000 (10.2-53.3)
HDL (mg/dl)	56.1	9.5	57.4	10.7	N/S
LDL (mg/dl)*	116.2	41.9	84.5	19.5	0.000 (11.7-45.6)
TG (mg/dl)	91.6	79.7	73.9	20.5	N/S
Apo A (mg/dl)	190.9	69.7	159.5	53.0	N/S
Apo B (mg/dl)*	126.8	50.4	93.0	26.1	0.000 (12.9-54.6)
Glucose (mg/dl)	83.4	6.2	85.4	5.9	N/S
Fibrinogen (mg/dl)*	350.2	108.0	287.5	91.5	N S
Homocysteine (µmol/l)	10.0	4.0	10.3	3.5	N S

* p: <0.05 Univariate Analysis of Variance

Conversion factor: 1mg dl cholesterol=0.025 mmol/l

Among patients the levels of total cholesterol correlated positively with age (p= 0.000 r=0.564 Pearson correlation) and the duration of the illness (p= 0.034, r=0.409). Total cholesterol and LDL correlated negatively with free T3 (p= 0.001 r= -0.435 and 0.003 r= -0.380 respectively), estradiol (p= 0.023 r= -0.292 and 0.014, r= -0.318 respectively) and insulin (p= 0.030 r= -0.280 and 0.037, r= -0.272 respectively). Free T4 also correlated negatively with total cholesterol (p= 0.016, r= -0.310) but not with LDL. The patients with hypercholesterolemia (TC >200 mg/dl) (N=11) had lower mean values of free T3 (3.9 pmol/l, SD 0.8), T4 (11.3 pmol/l, SD 2.6), estradiol (1.20 pmol/l SD: 1.00) and insulin (26.7 pmol/l, SD 16.6) compared with the patients with desirable cholesterol levels (N=19), whose values for free T3 were 4.7 pmol/l (SD 0.9), for T4: 12.8 pmol/l (SD 1.9), for estradiol: 1.98 pmol/l (SD 0.89) and for insulin: 45.2 pmol/l (SD 29.6).

Table 4.5: Hormone profile of patients on admission and controls adjusted for age and BMI (kg/m^2)

Hormones	**Patients with AN (N=30)**		**Control subjects (N=30)**		**P values**
	Mean	**SD**	**Mean**	**SD**	(95% confidence interval)
Free T3 (pmol/l)*	4.4	0.9	5.1	0.6	0.000 (4.5-4.9)
Free T4 (pmol/l)*	12.2	2.3	13.6	1.8	0.005 (12.4-13.4)
Free T4/T3 ratio*	2.9	0.6	1.3	1.6	0.000 (1.9-2.2)
Insulin (pmol/l)	38.5	26.8	40.5	10.0	N/S
Cortisol (µg/dl)	16.1	4.8	15.0	4.0	N/S
Estradiol (pmol/l)*	1.7	1.0	2.2	0.7	0.003 (1.7-2.1)

* p: <0.05 Univariate Analysis of Variance

Table 4.6: Lipid-soluble vitamins in patients on admission and controls adjusted for TC (Mean and SD)

Variables	Patients (N= 30)		Controls (N= 30)		P values
	Mean	SD	Mean	SD	(95% confidence interval)
Tocopherol mg/dl (µmol/l)*	1.0	0.3	1.2	0.8	0.009 (0.8-1.1)
Retinol µg/dl (µmol/l)*	46.9	13.0	68.2	29.9	0.003 (51.1-63.5)
α Carotene µg/dl	7.9	8.8	4.4	4.1	N/S
Carotene µg/dl (µmol/l)	23.8	26.4	11.8	11.5	N/S
Total carotenes µg/dl*	63.5	48.0	40.1	21.5	N/S
Lutein µg/dl*	17.7	15.5	12.0	6.6	0.005 (11.9- 17.8)
Crytoxanthine µg/dl*	6.1	5.0	4.1	2.8	0.004 (4.1-6.0)
Lycopene µg/dl	9.4	5.2	7.8	5.0	N/S

* p: <005 Univariate Analysis of Variance

Conversion factor: 1 mg/dl Tocopherol= 0.04307 µmol/l
1 µg/dl Retinol= 28.646 µmol/l
1 µg/dl Carotene= 53.689 µmol/l

Fatty acids

There were significantly higher proportions of EPA and total n-3 fatty acids in patients compared with controls. Although no significant values were found, there was a clear tendency of SFA and oleic acid proportions to be decreased in patients (Table 4.7).

4.3. Study 4. Comparison of cardiovascular risk factors, hormonal status and nutritional indices before and after treatment in patients with AN

4.3.1. Hypotheses

The risk factors for cardiovascular disease were expected to change following treatment of AN. The following hypotheses were tested:

- After treatment the altered biochemical parameters in patients with AN will be corrected to normal levels.

- After treatment the normalisation of the parameters is associated with indices of recovery based on the diagnostic criteria (BMI greater than 18.5 kg/m^2, normal menstruation, absence of purging behaviours, body dissatisfaction, and phobia of weight gain).

4.3.2. Methods

Of the thirty patients recruited on admission, two patients refused to participate in the follow up and seven patients dropped out of treatment. Consequently, twenty-one patients were re-tested at a 4.3 month of treatment (SD 0.9). Patients who dropped out of treatment had a significantly lower BMI on admission (mean: 16.9 kg/m^2, SD 2.2) than patients who were followed up (mean: 18.2 kg/m^2, SD 1.7).

In the twenty-one patients who were followed up, all the biochemical indices analysed in the first study were re-tested. Fibrinogen, Apo A and B concentrations were not determined at follow up because of cost considerations. Biochemical parameters were associated with the indices of recovery. These indices were based on the diagnosis criteria and include a BMI > 18.5 kg/m^2, menstruation, purging behaviours, body satisfaction and phobia of weight gain. This information was

Table 4.10: Fatty acid proportions and concentrations of plasma total lipids in patients on admission and follow-up (Mean and SD)

Fatty acid profile	Admission				Follow up				P value	
	Mean		SD		Mean		SD			
	%	Mmol/l	%	Mmol/l	%	mmol/l	%	Mmol/l	%	mmol/l
Palmitic acid (16: 0)	**19.44**	**2.69**	2.21	1.59	**18.67**	**2.21**	1.95	0.69	0.026	N/S
Palmitoleic acid (16: 1 n-7)	**2.46**	**0.47**	1.36	0.43	**2.44**	**0.31**	1.09	0.21	N/S	N/S
Stearic acid (18: 0)	**7.49**	**0.83**	0.76	0.31	**7.45**	**0.77**	0.86	0.14	N/S	N/S
Oleic acid (18: 1 n-9)	**18.03**	**2.28**	2.60	1.41	**17.74**	**1.91**	1.90	0.58	N/S	N/S
Linoleic acid (18: 2 n-6)	**32.27**	**3.40**	5.02	1.19	**31.70**	**3.34**	4.19	0.65	N/S	N/S
Gamma linolenic acid (18: 3 n-6)	**0.12**	**0.02**	0.02	0.01	**0.12**	**0.01**	0.02	0.00	N/S	N/S
Alpha linolenic acid (18: 3 n-3)	**0.37**	**0.03**	0.12	0.03	**0.39**	**0.04**	0.18	0.02	N/S	N/S
Mead acid (20: 3 n-9)	**0.15**	**0.03**	0.04	0.03	**0.16**	**0.02**	0.04	0.01	N/S	0.010
Dihomogamma linolenic acid (20:3n-6)	**1.76**	**0.01**	0.55	0.09	**1.79**	**0.17**	0.50	0.06	N/S	0.009
Arachidonic acid (20: 4 n-6)	**6.67**	**0.45**	1.19	0.30	**7.13**	**0.70**	1.08	0.17	0.018	0.005
EPA (20: 5 n-3)	**0.45**	**0.35**	0.17	0.39	**0.52**	**0.05**	0.25	0.03	N/S	0.003
7,10,13,16-Docosatetraenoic (22: 4 n-6)	**0.61**	**0.05**	0.09	0.02	**0.61**	**0.05**	0.08	0.01	N/S	N/S
4,7,10,13, 16-Docosapentaenoic (22: 5 n-6)	**0.53**	**0.05**	0.13	0.02	**0.51**	**0.04**	0.13	0.01	N/S	N/S
DPA (22: 5 n-3)	**0.45**	**0.04**	0.12	0.01	**0.47**	**0.04**	0.09	0.01	N/S	N/S
DHA (22: 6 n-3)	**1.39**	**0.04**	0.58	0.01	**1.74**	**0.15**	0.75	0.07	0.002	0.000
Total SFA	**29.40**	**3.44**	3.15	1.78	**28.55**	**3.29**	2.79	1.00	N/S	0.000
Total PUFA	**44.86**	**4.53**	5.20	1.29	**44.97**	**7.77**	4.35	1.69	N/S	0.000
Ratio Mead/Arachidonic (20: 3 n-9/20: 4 n-6)	**0.02**	**0.02**	0.01	0.01	**0.02**	**0.02**	0.01	0.01	N/S	N/S
Total n-3	**2.61**	**0.24**	0.68	0.07	**3.01**	**0.29**	1.19	0.11	N/S	N/S
Total n-6	**42.10**	**4.27**	5.05	1.24	**41.80**	**4.33**	4.42	0.81	N/S	N/S
Ratio n-6/n-3	**17.04**	**18.28**	4.35	4.27	**16.43**	**16.67**	8.17	5.63	N/S	N/S

Paired t-test $p<0.01$